Microbial diversity in mine tailings and the role of metal sulfide oxidizers in biomining processes

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Zusammenfassung

Diese Arbeit beschreibt die Vielfalt azidophiler Eisen- und Schwefel-oxidierender Mikroorganismen in verschiedenen sauren und sulfidischen Bergehalden (mine tailings) und ihre Rolle bei der Metall(Kupfer)-Biolaugung unter Einsatz Kultivierung-abhängiger und -unabhängiger Methoden.

Erstens wurde die Zusammensetzung mikrobieller Gemeinschaften sowie biogeochemische Prozesse in einer mehrfach extremen Bergehalde (tailings) an der Meeresküste in der Bucht von Chañaral in der Atacama-Wüste, Chile untersucht. Die Bergehalde war nahezu frei von organischem Kohlenstoff und charakterisiert durch arides Klima, niedrigen pH-Wert, hohen Salzgehalt und hohe Konzentrationen an löslichen Metallen aufgrund von Metallsulfid(Pyrit)-Oxidation. Die Ergebnisse zeigten, dass die wahrscheinlich bisher extremste untersuchte Bergehalde von Mikroorganismen mit Gesamtzellzahlen von bis zu 10^8 Zellen g^{-1} besiedelt war. Eine real-time PCR Quantifizierung ergab eine Dominanz der Bakterien über Archaeen. Die Zusammensetzung der mikrobiellen Gemeinschaften analysiert mittels 16S rRNA Gen-Sequenzierung über Klonbibliotheken war an verschiedenen Probenahmestellen und Tiefen sehr variabel. Pyritoxidation und die Freisetzung von Metallionen wurden durch azidophile Eisen- und Schwefel-oxidierende Bakterien der Gattungen *Acidithiobacillus*, *Alicyclobacillus* und *Sulfobacillus* angetrieben. Die Anwesenheit von Eisen-Oxidierern und ihrer Aktivität auch bei hohen Salzgehalten von bis zu 1 M NaCl wurde bestätigt mittels real-time PCR, 16S rRNA Gen-Sequenzierung, Kultivierung, sowie Mikrokalorimetrie. Das Vorkommen der Eisen–Oxidierer korrellierte mit Maxima des Pyritgehaltes und der biologischen Pyritoxidationsrate.

Zweitens wurde im Gegensatz zu Bergehalden bei pH<3 das gemäßigt saure Stadium in Bergehalden bislang kaum untersucht. Daher wurden die mikrobiellen Gemeinschaften von drei verschiedenen sulfidischen und sauren Bergehalden in Botswana, Deutschland und Schweden im pH-Bereich von 3,2 bis 6,5 mittels 16S rRNA Gendiversitätsanalyse über Klonbibliotheken analysiert. Die phylogenetischen Analysen zeigten, dass die bakteriellen Sequenzen den sechs Phyla *Proteobacteria*, *Firmicutes*, *Nitrospira*, *Actinobacteria*, *Acidobacteria* und *Bacteroidetes* zuzuordnen waren. Am häufigsten traten in den Klonbibliotheken Vertreter der *Proteobacteria* und *Firmicutes* auf, allerdings variierte die Zusammensetzung der mikrobiellen Gemeinschaften stark zwischen den drei Standorten und in verschiedenen Tiefen, vor allem aufgrund der pH-Differenzierung.

Drittens, um die Aktivität der azidophilen Eisen- und Schwefel-Oxidierer mit unterschiedlichen Temperaturoptima bei der Kupfer-Biolaugung zu untersuchen, wurden vergleichende Batch-Kultur Biolaugungs-Experimente durchgeführt. Dabei wurden ein marines, hydrothermales polymetallisches Sulfid-Erz, mit überwiegend Kupferkies als Substrat, sowie mesophile, moderat thermophile oder thermophile azidophile Eisen- und Schwefel-oxidierende Bakterien und Archaeen eingesetzt. Die Ergebnisse zeigten die effektivste Kupfer-Biolaugung mit dem thermophilen *Acidianus brierleyi*.

Abstract

This thesis describes the diversity of acidophilic iron- and sulfur-oxidizing prokaryotes in different acidic and sulfidic mine tailings and their role in metal (copper) bioleaching using cultivation-dependent and -independent methods.

Firstly, the microbial community composition and biogeochemical processes in a multiple extreme marine shore copper mine waste tailings dump at Chañaral Bay in the Atacama Desert, Chile were studied. The almost organic-carbon free mine tailings dump was characterized by arid climate, low pH, high salinity and high soluble metal concentrations due to metal sulfide (pyrite) oxidation. The results showed that the likely most extreme mine tailings studied so far was colonized by microorganisms with total cell numbers of up to 10^8 cells g^{-1}. Real-time PCR quantification revealed a dominance of bacteria over archaea. The composition of the microbial communities analyzed by 16S rRNA gene sequencing via clone libraries was highly variable at different sampling sites and depths. Pyrite oxidation and the release of metal ions were driven by acidophilic iron- and sulfur-oxidizing bacteria of the genera *Acidithiobacillus*, *Alicyclobacillus*, and *Sulfobacillus.* The presence of iron-oxidizers and their activity even at high salinity of up to 1 M NaCl was confirmed by real-time PCR, 16S rRNA gene sequencing, cultivation, as well as microcalorimetry. The abundance of the iron-oxidizers coincided with maxima of the pyrite content and biological pyrite oxidation rates.

Secondly, in contrast to tailings at pH < 3 the moderate acidic stage in mine tailings is only scarcely studied. Thus, the microbial communities of three different sulfidic and acidic mine waste tailing dumps in Botswana, Germany and Sweden at the pH range of 3.2 to 6.5 were analyzed by 16S rRNA gene diversity analysis via clone libraries. The phylogenetic analyses showed that the bacterial sequences clustered in the six phyla Proteobacteria, Firmicutes, Nitrospira, Actinobacteria, Acidobacteria and Bacteroidetes. Most abundant in the clone libraries were representatives of the phyla Proteobacteria and Firmicutes, however the microbial community composition strongly differed between the three sites and at different tailings depths mainly due to pH differentiation.

Thirdly, to examine the activity of acidophilic iron- and sulfur oxidizers with different temperature optima on copper bioleaching, comparative batch culture bioleaching experiments were carried out. These contained a marine hydrothermal polymetallic sulfide ore containing mainly chalcopyrite as substrate as well as mesophilic, moderate thermophilic or thermophilic acidophilic iron- and sulfur- oxidizing bacteria and archaea. The results showed a most effective copper bioleaching with the thermophile *Acidianus brierleyi*.

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Abbreviations

ARD/AMD	acid rock drainage / acid mine drainage
ATP	adenosine triphosphate
CARD-FISH	catalyzed reporter deposition - fluorescence in situ hybridization
Cyc2	cytochrome *c*2
dsr	dissimilatory sulfite reductase gene
EPS	extracellular polymeric substances
FISH	fluorescence in situ hybridization
IOPs	iron-oxidizing prokaryotes
ISOPs	iron- and sulfur-oxidizing prokaryotes
MHS	marine hydrothermal sulfide
MS	metal sulfide
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
PCR	polymerase chain reaction
q-PCR	quantitative polymerase chain reaction
RISCs	reduced inorganic sulfur compounds
rRNA	ribosomal ribonucleic acid
SGDC	SYBR-Green direct counts
SOPs	sulfur-oxidizing prokaryotes
$T_{op.}$	optimum temperature

Chapter 1

Introduction

1. Introduction

1.1. Acidophilic chemolithotrophic iron- and sulfur-oxidizing prokaryotes (ISOPs)

The ability of certain prokaryotes to use energy from inorganic substrates, in the absence of light, was described for the first time in the late 19th century (Winogradsky, 1887). Nowadays these microorganisms are known as chemolithotrophs that are extraordinary diverse in respect of the utilization of different chemical substrates, modes of carbon nutrition, morphology and habitat. As the term provides, chemolithotrophs obtain sufficient energy to support ATP synthesis and electron transport from the oxidation of different inorganic substrates such as sulfur and iron (Dopson and Johnson, 2012; Kim and Gad, 2008; Kelly and Wood, 2006) and can utilize carbon from carbon dioxide (CO_2) (chemolithoautotrophs) or from organic sources (chemolithoheterotrophs) for cell biosynthesis and maintenance (Figure 1).

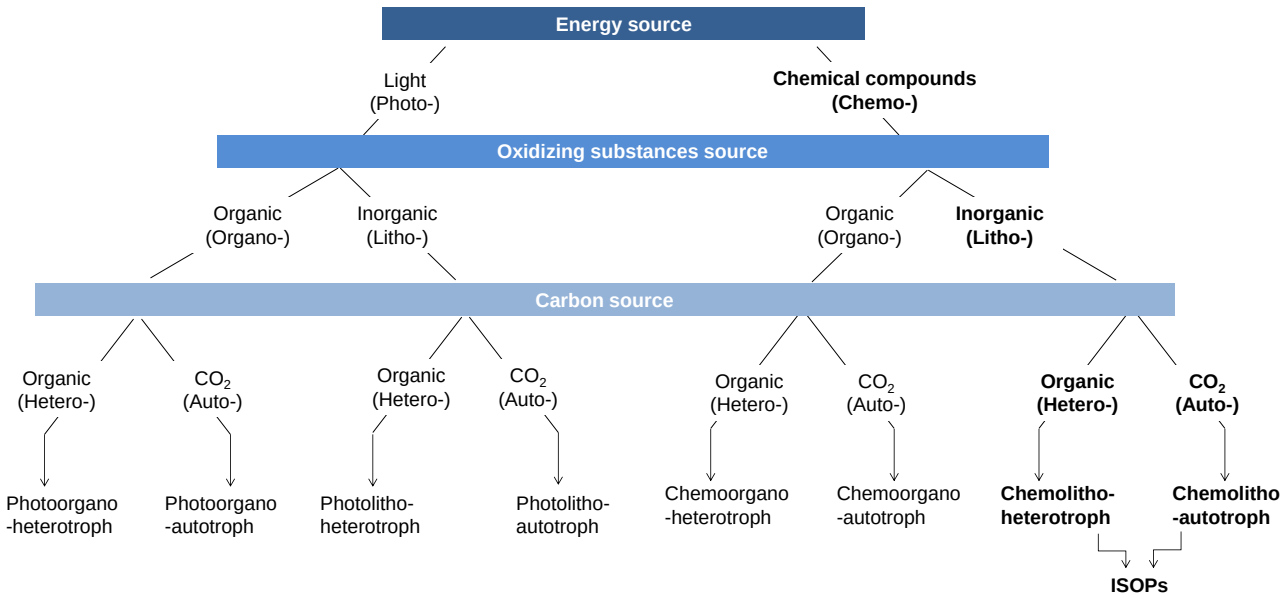

Figure 1. Overview of metabolic characteristic of microorganisms and particularly acidophilic iron- and sulfur-oxidizing prokaryotes (ISOPs).

Iron- and sulfur-oxidizing prokaryotes (ISOPs) occur in moderate to extreme acidic environments such as volcanic and geothermal areas (natural environment) or metal sulfide mines (man-made environment). They play key roles in determining the geochemistry of the environments through the dissimilatory oxidation of reduced inorganic sulfur compounds (RISCs) and/or iron and consequently generating sulfuric acid. This acid and the oxidized form of iron can further solubilize ores that results in releasing elements such as metals from ores. Therefore, acidophilic ISOPs are economically and ecologically important, due to their role in oxidizing metal sulfides (MS) and mobilizing metals (biomining) as well as generating acidic waters (acid mine/rock drainage) that contaminate the environment (Hallberg and Johnson, 2001).

1.1.1. Oxidation of sulfur and RISCs by acidophilic sulfur-oxidizing prokaryotes (SOPs)

Acidophilic sulfur-oxidizing prokaryotes (SOPs) are primarily Gram-negative bacteria which can obtain some or all of their energy requirements from the oxidation of elemental sulfur (S^0) and/or various reduced inorganic sulfur compounds (RISCs) such as sulfide (S^{2-}), thiosulfate ($S_2O_3^{2-}$), tetrathionate ($S_4O_6^{2-}$), trithionate ($S_3O_6^{2-}$) and sulfite (SO_3^{2-}) (Friedrich et al., 2001) by acid generating reactions (Equations 1 to 4) (Vidyalakshmi et al., 2009; Kelly and Wood, 2006). Besides oxygen as a final electron acceptor (Equations 1 and 2), some acidophilic SOPs are also able to grow anaerobically using oxidized iron (Fe^{3+}) and nitrogen compounds (e.g., nitrate) as electron acceptor (Equation 3 and 4) (Johnson and Hallberg, 2009; Robertson and Kuenen, 2006; Kelly and Wood, 2006).

$$S^0 + H_2O + 1.5\ O_2 \xrightarrow{SOP} SO_4^{2-} + 2\ H^+ \qquad \text{(eq. 1)}$$

$$S_2O_3^{2-} + 2\ O_2 + H_2O \xrightarrow{SOP} 2\ SO_4^{2-} + 2\ H^+ \qquad \text{(eq. 2)}$$

$$S^0 + 4\ H_2O + 6\ Fe^{3+} \xrightarrow{SOP} SO_4^{2-} + 8\ H^+ + 6\ Fe^{2+} \quad \text{(eq. 3)}$$

$$S^0 + 3\ NO_3^- + H_2O \xrightarrow{SOP} SO_4^{2-} + 2\ H^+ + 3\ NO_2^- \quad \text{(eq. 4)}$$

The first described acidophilic SOPs were *Acidithiobacillus (At.) thiooxidans* (Waksman and Joffe, 1922) in the domain *Bacteria* and *Sulfolobus acidocaldarius* (De Rosa et al., 1975) in the domain *Archaea*. Acidophilic SOPs are phylogenetically diverse and belong to the genera *Acidiferrobacter*, *Acidithiobacillus* (formerly *Thiobacillus*), *Acidiplasma*, *Acidianus, Alicyclobacillus*, *Metallosphaera*, *Sulfobacillus*, *Sulfolobus*, *Sulfurcoccus, Desulfurolobus* (Schippers et al., 2014; Kim and Gadd, 2008; Robertson and Kuenen, 2006; Kelly and Wood, 2000; Brock et al., 1972). However, in addition to acidophilic SOPs relevant for the oxidation of metal sulfides (see Table 1 below) there are neutrophilic or moderate acidophilic SOPs of e.g. the genera *Halothiobacillus, Thiobacillus,* or *Thiomonas* that are usually not found in extremely acidic environments (Dopson and Johnson, 2012).

1.1.1.1. Biochemistry of sulfur and RISCs oxidation

The biochemistry of sulfur and RISCs oxidation is very complex and various models have been identified in different neutrophilic or acidophilic SOPs. In acidophilic mesophilic SOPs such as *At. thiooxidans* and *At. ferrooxidans*, sulfur and RISCs oxidation takes place in periplasmic space or in the cytoplasm. Consequently, extracellular elemental sulfur has to pass the outer membrane prior to oxidation. As only highly reactive thiol-bound sulfane sulfur atoms (R-S-SH) but not sulfide or elemental sulfur can pass the outer membrane to be oxidized, thiol group-contain (R-SH) proteins are employed in the outer membrane and periplasmic space for sulfur transport. Then the sulfane sulfur is oxidized by e.g. the sulfur diooxygenase (*SDO*)

and the thiol groups are set free again. The product of the dioxygenase reaction is sulfite which is mainly oxidized further to sulfate by sulfite acceptor oxidoreductase (*SAOR*). In the case of thiosulfate ($S_2O_3^{2-}$) formation in periplasmic space, thiosulfate quinone oxidoreductase (*TQO*) oxidizes thiosulfate to tetrathionate ($S_4O_6^{2-}$), then tetrathionate is hydrolysed by tetrathionate hydrolase (*TetH*) and converted to thiosulfate and other products. In addition, sulfide quinone reductase (*SQR*) which is responsible for oxidizing hydrogen sulfide as well as rhodanese and thiosulfate sulfurtransferase (*TST*) have been identified. *SQR*, *TQO* and the cytoplasmic heterodisulfide reductase complex (*HdrABC*) enzymes have been demonstrated to be active during sulfur oxidation in *At. ferrooxidans*. However, the sulfide:Fe(III)ion and sulfite:Fe(III)ion oxidoreductase activities have been also described in *At. ferrooxidans* as iron- and sulfur-oxidizing bacteria. In *At. ferrooxidans,* electrons from the oxidation of RISCs are transferred via the quinol pool (*QH2*) to terminal oxidases or to NADH complex I to produce ATP or NADPH, respectively. The RISCs oxidation system is different in *A. caldus* from that in *At. ferrooxidans* and *At. thiooxidans*. The sulfur oxidation system of *A. caldus* can be classified into three subsystems: the truncated *Sox* subsystem, non-*Sox* sulfur subsystem, and *SOR* subsystem. The truncated *Sox* subsystem converts both sulfur atoms of thiosulfate to sulfate without the formation of any intermediate. The non-*Sox* sulfur subsystem, similar to the sulfur oxidation system in *At. ferrooxidans*, contains the sulfur oxidation enzyme genes (*TetH*, *SQR*, and *HdrABC*) and terminal oxidase genes. The *SOR* subsystem is characterized by the sulfur oxygenase reductase gene (*SAOR*) in *A. caldus*, which was only found in several acidophilic and thermophilic archaea but not in the two species *At. ferrooxidans* and *At. thiooxidans* (Dopson and Johnson, 2012; Chen et al., 2012; Ghosh and Dam, 2009; Rohwerder and Sand, 2007; Friedrich et al., 2001).

1.1.2. Oxidation of iron by acidophilic iron-oxidizing prokaryotes (IOPs)

Since ferrous iron (Fe^{2+}) is chemically oxidized very slowly by molecular oxygen at pH<4, acidophilic iron-oxidizing microorganisms (IOMs) have a main role in iron oxidation in acidic environments. A variety of IOMs are known to catalyse the oxidation of ferrous iron (Table 1) to ferric iron (Equation 5) and only a few of them oxidize it obligatory (Schippers et al., 2014; Johnson, 2012; Hedrich et al., 2011; Johnson and Hallberg, 2009; Johnson, 2006).

$$Fe^{2+} + 0.25\ O_2 + H^+ \xrightarrow{IOP} Fe^{3+} + 0.5\ H_2O \qquad \text{(eq. 5)}$$

Obligate acidophilic IOMs belong to the Gram-negative *Leptospirillum ferrooxidans*, *L. ferriphilum* and ‘‘*L. ferrodiazotrophum*’’. *Acidithiobacillus ferrooxidans, At. ferridurans* or *At. ferrivorans* are facultative iron-oxidizers and also oxidize RISCs. Growth on hydrogen was observed for *At. ferrooxidans* and *At. ferridurans* but not for *At. ferrivorans*. *Acidithiobacillus* spp. can also grow anaerobically using ferric iron as an electron acceptor (Schippers et al. 2014; Hedrich and Johnson, 2013; Hedrich et al., 2011; Johnson and Hallberg, 2009).

The dominance of *Acidithiobacillus* spp. and *Leptospirillum* spp. is different in acidic environments due to their physiological differences. *At. ferrooxidans* has higher specific iron oxidation activity than *L. ferrooxidans* at low redox potentials (e.g. high Fe^{2+}/Fe^{3+} ratios) and cannot oxidize iron above a redox potential of +840 mV, but *L. ferrooxidans* is less inhibited by Fe^{3+}. Therefore, *Leptospirillum* spp. are dominant where Fe^{3+} concentration are high and *At. ferrooxidans* dominates in acidic systems with high concentration of Fe^{2+} (Johnson et al., 2012; Johnson and Hallberg, 2009). Besides obiligate iron-oxidizers *Leptosprillum* spp., facultative iron-oxidizers

Sulfobacillus spp., *Alicyclobacillus* spp. and *Acidimicrobium ferrooxidans* are also known iron-oxidizers (Johnson and Hallberg, 2009).

1.1.2.1. Biochemistry of iron oxidation

As the free energy associated with the oxidation of iron is rather small (only one mole of electrons is related per mole Fe^{2+} oxidized) and the Fe^{2+}/Fe^{3+} couple redox potential (+770 mV) is close to that of oxygen/water couple (+820 mV) at low pH, growth yields on ferrous iron are low (Johnson et al., 2012).

During the oxidation of ferrous to ferric iron, electrons are used to reduce oxygen and protons to water. Not all electrons, however, are used for the reduction of oxygen in autotrophic iron-oxidizers. Electrons reduced NAD(P) to NAD(P)H which is required for carbon dioxide fixation and other cellular metabolic functions. In the case of ferrous iron, where the redox potential is more positive than that of the NAD(P)/NAD(P)H couple, electrons must be transported uphill by the electron transport chain to NAD(P). It has been hypothesized that this occurs by reverse electron transport through the cytochrome bc_1 complex, the quinone pool and the NAD(P)H dehydrogenase, and is energized by the proton-motive force generated by the hydrolysis of ATP. For this reason, growth yields of IOMs that use ferrous iron as sole energy source are low.

The characterization of the electron transport chain of the iron-oxidizer *At. ferrooxidans* provided evidence for the existence of a bc_1 complex. Electron transport via the bc_1 complex was accelerated by addition of ATP. While no similar direct evidence for the uphill electron transport has been provided in other iron-oxidizing autotrophs, genes coding for similar uphill electron transport chain components have been detected in the reconstructed genomes of *Leptospirillum* species found in Iron Mountain,

California, and were found to be expressed *in situ* (Ram et al., 2005; Tyson et al., 2004).

For many years, it has been known that the blue copper protein rusticyanin is involved in transporting of electrons from ferrous iron to oxygen in *At*. *ferrooxidans*. An acid-stable cytochrome *c* that was able catalysing the ferrous iron-dependent reduction of rusticyanin suggested that this cytochrome *c* is the actual electron acceptor from ferrous iron. The surveys have shown that cytochrome *c* (Cyc2) is actually a membrane-bound protein that is most likely to be the oxidant of ferrous iron. Cytochrome *c* is known in *At. ferrooxidans*, but information about iron-oxidation enzymes in other iron-oxidizers is relatively scarce (Bonnefoy and Holmes, 2011; Johnson and Hallberg, 2009).

1.1.3. Diversity of acidophilic ISOPs

Acidophilic ISOPs represent an extremely diverse group with respect to their phylogeny, physiology and ecology. Temperature has a determinative effect on acidophilic ISOPs activity and consequently oxidation processes. Therefore, the ISOPs are divided on the basis of their optimum growth temperature into three groups: i) mesophiles, ii) thermotolerant/moderate thermophiles and iii) thermophiles. Mesophiles and most moderate thermophiles included species occuring in the domain *Bacteria* while thermophiles belong to the domain *Archaea* (Johnson et al., 2012; Schippers, 2007; Johnson, 1998).

1.1.3.1. Mesophilic acidophilic ISOPs

The most studied acidophilic ISOPs belong to the mesophiles which have an optimum temperature about or more often below 40˚C (Ding et al., 2011; Johnson, 1998).

Mesophiles belong to the phyla *Proteobacteria*, *Nitrospira*, *Actinobacteria* and *Firmicutes* (Schippers et al., 2014; Schippers, 2007). Some of these are psychrotolerant.

Acidithiobacillus spp. is the most described mesophilic ISOPs in the phylum *Proteobacteria* that is assigned to the class *Acidithiobacillia* (formerly class *Gammaproteobacteria*; Kelly et al., 2013). All species of the genus *Acidithiobacillus* comprising *At. ferrooxidans*, *At. thiooxidans*, *At. albertensis, At. ferrivorans, At. ferridurans* are mesophiles excluding *At. caldus* (Table 1; Schippers et al., 2014; Hedrich and Johnson, 2013; Hallberg et al., 2010; Schippers, 2007). In addition, *Thiobacillus prosperus* and *Thiobacillus plumbophilus* are also acidomesophilic chemolithotrophic *Proteobacteria* but no significant homology to *Acidithiobacillus* species was detected by DNA-DNA hybridization (Schippers, 2007). The other mesophilic species in the phylum *Proteobacteria* are moderate acidophilic *Thiomonas cuprina* that grow facultatively chemolitotrophic and mixotrophic (Schippers, 2007) and the iron and sulfur oxidizer *Acidiferrobacter thiooxydans* (formerly *Thiobacillus ferrooxidans* m-1) which grow optimally at 38°C (Schippers et al., 2014; Hallberg et al., 2011). The betaproteobacterium ”*Ferrovum myxofaciens”* is an extreme acidophilic obligate iron-oxidizer growing as macroscopic streamers in rivers or streams (Hedrich et al., 2011).

In phylum *Nitrospira*, the genus *Leptospirillum* was validly described. *Leptospirillum* spp. are obligate acidophilic (pH<4) and comprise the following species: *L. ferrooxidans* (group I), *L. ferriphilum* (group II) and ‘‘*L. ferrodiazotrophum*” (group III) (Tyson et al., 2005). *Leptosprillum ferriphilum* is a thermotolerant mesophilic iron-oxidizer which can grow at up to 45˚C (Schippers et al., 2014; Schippers, 2007).

In phylum *Actinobacteria*, *Ferrimicrobium acidiphilum* is a mesophilic heterotrophic iron-oxidizer that does not grow on sulfur compounds. In phylum *Firmicutes, Alicyclobacillus disulfidooxidans* and ‘‘*Sulfobacillus montserratensis*’’ have been described as mesophiles while *Alicyclobacillus tolerans* and *Sulfobacillus thermotolerans* are thermotolerant mesophilic iron- and/or sulfur-oxidizing bacteria (Schippers et al., 2014; Schippers, 2007). Moreover, *Sulfobacillus benefaciens* is a thermotolerant mesophilic iron- and/or sulfur-oxidizing bacterium which can grow via ferric iron respiration in the absence of oxygen (Schippers et al., 2014; Johnson et al., 2008).

In addition to mesophilic bacteria, mesophilic iron-oxidizing archaea belong to the genus *Ferroplasma* within the phylum *Euryarchaeota*. The genus *Ferroplasma* comprises the following species: mesophilic *Ferroplasma acidiphilum* and thermotolerant mesophilic ‘‘*Ferroplasma acidarmanus*’’. Both species can grow aerobically on ferrous iron (Fe^{2+}) as well as anaerobically on ferric iron (Fe^{3+}) (Schippers et al., 2014; Schippers, 2007).

1.1.3.2. Moderate thermophilic acidophilic ISOPs

Moderate thermophiles have an optimum temperature ($T_{op.}$) above 45˚C (up to 60 °C). In the domain *Bacteria*, *A. caldus* (*Proteobacteria*), *Acidimicrobium ferrooxidans*, *Acidimicrobium* P1/P2 and *Ferrithrix thermotolerans* (*Actinobacteria*), *Sulfobacillus acidophilus*, *Sulfobacillus sibiricus* and *Sulfobacillus thermosulfidooxidans* (*Firmicutes*) are chemolithotrophic moderate thermophilic bacteria (Schippers et al., 2014; Schippers, 2007). *Ferrithrix thermotolerans* is obligately heterotrophic and able to oxidize and reduce iron but not able to oxidize sulfur compounds (Schippers et al., 2014; Johnson et al., 2009).

In the domain *Archaea*, the moderate thermophilic *Acidiplasma cupricumulans* (formerly *Ferroplasma cupricumulans*; Golyshina et al., 2009) can oxidize iron and sulfur (Schippers et al., 2014).

1.1.3.3. Thermophilic acidophilic ISOPs

Thermophilic iron and/or sulfur oxidizing *Archaea* belong to the phylum *Crenarchaeota* and to the family *Sulfolobaceae* which can grow at above 60˚C. The thermophilic genera *Sulfolobus* spp. (*S. metallicus*, *S. yangminggensis*), *Sulfurococcus* spp. (*S. mirabilis*, *S. yellowstonensis*), *Acidianus* spp. (*Ac. brieleyi*, *Ac. infernus* and *Ac. sulfidivorans*), and *Metallosphaera* spp. (*M. hakonensis*, *M. prunae* and *M. sedula*) are able to oxidize iron and sulfur at high temperature (Table 1) (Schippers et al., 2014; Ding et al., 2011; Gonzalez-Contreras and Weijma, 2011; Schippers, 2007; Huber and Stetter, 1989; Segerer et al., 1986).

1.2. Metal sulfide oxidation by acidophilic ISOPs

1.2.1. Metal sulfides (MS)

Metal sulfides (MS) are minerals that are commonly found in terrestrial rocks and contain as major elements sulfur and metals such as Fe, Cu, Zn, Pb, Ag and others. There are several hundred known sulfide minerals, but only a half dozen of them such as pyrite (FeS_2), chalcopyrite ($CuFeS_2$), pyrrhotite $Fe_{(1-x)}S$ (x= 0 to 0.2), sphalerite ZnS, chalcocite (CuS_2) and galena (PbS) are sufficiently abundant to be regarded as ‘‘rock-forming minerals’’ which occur mostly as sub-minerals in certain major rock types. These sulfide minerals are enriched in ores increasingly mined to meet the world demand for metals such as copper. However, a very large volume of extracted rock only contains a few percent (commonly less than one percent) of the desired

metal. In addition to terrestrial sulfidic mines (underground and open pit), metal sulfides formed in hydrothermal systems in the deep oceans are also taken into consideration for mining in recent years.

Table 1. Optimum temperature and iron- and/or sulfur-oxidizing ability of acidophilic ISOPs. op.= Optimum, ox.=Oxidation, na= Data not available (from Schippers et al., 2014, modified).

Species	Temperature $_{op.}$ (°C)	Domain/Phylum	$Fe_{Ox.}$	$S_{Ox.}$
Mesophiles				
Acidiferrobacter thiooxydans	38	Bacteria/Proteobacteria	+	+
Acidithiobacillus ferrooxidans	30-35	Bacteria/Proteobacteria	+	+
Acidithiobacillus thiooxidans	28-30	Bacteria/Proteobacteria	-	+
Acidithiobacillus ferrivorans	27-32	Bacteria/Proteobacteria	+	+
Acidithiobacillus ferridurans	29	Bacteria/Proteobacteria	+	+
Acidithiobacillus albertensis	25-30	Bacteria/Proteobacteria	-	+
"*Thiobacillus prosperus*"	33-37	Bacteria/Proteobacteria	+	+
"*Thiobacillus plumbophilus*"	27	Bacteria/Proteobacteria	-	+
Thiomonas cuprina	30-36	Bacteria/Proteobacteria	-	+
Leptospirillum ferrooxidans	28-30	Bacteria/Nitrospira	+	-
Leptospirillum ferriphilum	30-37	Bacteria/Nitrospira	+	-
"*Leptospirillum ferrodiazotrophum*"	37	Bacteria/Nitrospira	+	na
Ferrimicrobium acidiphilum	35	Bacteria/Actinobacteria	+	-
Alicyclobacillus disulfidooxidans	35	Bacteria/Firmicutes	+	+
Alicyclobacillus tolerans	37-42	Bacteria/Firmicutes	+	+
Sulfobacillus benefaciens	38.5	Bacteria/Firmicutes	+	+
"*Sulfobacillus montserratensis*"	37	Bacteria/Firmicutes	+	+
Sulfobacillus thermotolerans	40	Bacteria/Firmicutes	+	+
"*Ferroplasma acidarmanus*"	42	Archaea/Euryarchaeota	+	-
Ferroplasma acidiphilum	35	Archaea/Euryarchaeota	+	-
Moderate thermophiles				
Acidithiobacillus caldus	45	Bacteria/Proteobacteria	-	+
Acidimicrobium ferrooxidans	45-50	Bacteria/Actinobacteria	+	-
"*Acidithiomicrobium* P1/P2"	50	Bacteria/Actinobacteria	+	+
Ferrithrix thermotolerans	43	Bacteria/Actinobacteria	+	-
Sulfobacillus acidophilus	45-50	Bacteria/Firmicutes	+	+
Sulfobacillus sibiricus	55	Bacteria/Firmicutes	+	+
Sulfobacillus thermosulfidooxidans	45-48	Bacteria/Firmicutes	+	+
Acidiplasma cupricumulans	54	Archaea/ Euryarchaeota	+	+
Thermophiles				
Acidianus brierleyi	~70	Archaea/ Crenarchaeota	+	+
Acidianus infernus	~90	Archaea/ Crenarchaeota	+	+
Acidianus sulfidivorans	74	Archaea/ Crenarchaeota	+	+
Metallosphaera hakonensis	70	Archaea/ Crenarchaeota	na	+
Metallosphaera prunae	~ 75	Archaea/ Crenarchaeota	+	+
Metallosphaera sedula	75	Archaea/ Crenarchaeota	+	+
Sulfolobus metallicus	65	Archaea/ Crenarchaeota	+	+
Sulfolobus yangmingensis	80	Archaea/ Crenarchaeota	na	+
Sulfurococcus yellowstonensis	60	Archaea/ Crenarchaeota	+	+
Sulfurococcus mirabilis	70-75	Archaea/ Crenarchaeota	+	+

In addition to raw materials, metal sulfide minerals are relevant from an environmental point of view since metal sulfides react with oxygen and water and generate acid rock/mine drainage (ARD/AMD) which can accelerate the dissolution of associated

minerals containing potentially toxic elements (e.g. As, Pb, Cd, etc.) (Dold, 2010). Since acidophilic ISOPs gain their energy by oxidizing the sulfur or the metal (in case of iron and copper) of sulfide minerals, metal sulfide dissolution and consequently ARD/AMD generation is enhanced by biological processes (Auld et al., 2013; Hallberg, 2010; Hao et al., 2010; Kim et al., 2009; Benner et al., 2000; Edwards et al., 1999; Gray, 1997).

1.2.2. Oxidation of metal sulfide minerals by ISOPs

Metal sulfide oxidation is a process in which microorganisms such as iron- and sulfur-oxidizers play an important role by promoting mineral dissolution. Although the chemical oxidation of metal sulfide minerals has been long recognized by geochemists, the role of acidophilic ISOPs that catalyse the oxidation process by mediating redox transformation of iron and sulfur were studied in recent decades (Schippers et al. 2014; Vera et al. 2013; Templeton, 2011; Rohwerder and Sand, 2007; Johnson, 2006; Weber et al., 2006; Gonzalez-Toril et al., 2003). Redox transformations occur in two different forms; changes of oxidation state; e.g. iron oxidation and/or phase changes; e.g. convert insoluble form of sulfides to soluble form of sulfate. The solubilisation process is termed bioleaching and occurs naturally wherever suitable conditions are found for the growth of acidophilic ISOPs. Various factors such as activity of microorganisms, bacterial concentrations, pH and ferric iron concentration, supply of oxygen and etc. are affecting the oxidation reactions of metal sulfides. The iron- and sulfur-oxidation rates differ from strain to strain and with different conditions. For instance, most acidophilic ISOPs are able to grow in the presence of various kinds of metal ions despite the toxic effect of them. Moreover, the ability of some acidophilic ISOPs to oxidize iron in the presence of high concentration

of chloride (sodium chloride) has been determined (Gahan et al., 2010; Huber and Stetter, 1989). The mentioned factors are affected significantly by pH of the growth medium. Most acidophilic ISOPs are unable to initiate growth on ferrous iron at pH>3. The growth of the bacteria is usually initiated at a very low pH range and as the growth continues in batch culture the pH of the medium increases and results in precipitating of ferric iron. A High concentration of ferric iron has an inhibiting effect on ferrous iron oxidation which can be observed in the growth of *Acidithiobacillus* spp. Besides all these factors, temperature has an effective role.

1.3. Metal sulfide oxidizing prokaryotes: industrial and environmental perspective

The use of acidophilic ISOPs to extract metals from metal sulfide ores is simply the harnessing of a natural process for commercial purpose. Indeed acidophilic ISOPs are either important for the biogeochemical iron and sulfur cycles or are important in geobiotechnical processes of metal bioleaching which is termed as biomining (Schippers et al., 2014; Brierley and Brierley 2013; Vera et al. 2013; Emerson et al., 2010; Kappler and Newman, 2003). The use of acidophilic ISOPs has some distinct advantages over the traditional physicochemical methods (hydrometallurgy and pyrometallurgy). Almost without exception, microbial extraction procedures are more environmentally friendly. They do not require high amounts of energy used during roasting or smelting and do not produce sulfur dioxide or other environmentally harmful gaseous emissions. However, acidophilic ISOPs can also mediate oxidation processes in mine waste rocks and mine tailings and thereby producing unwanted acid and metal pollution which is known as acid mine drainage and/or acid rock drainage (AMD/ARD).

1.3.1. Industrial perspective (biomining): recovery of metals (copper) via bioleaching

Biomining is the utilisation of biohydrometallurgy to process metal ores. Biohydrometallurgy is essentially the application of geobiotechnology to processing minerals. Therefore, biomining is technically a branch of hydrometallurgy, but uniquely it involves the use of microorganisms to generate chemical oxidants, such as ferric iron (Fe^{3+}) and protons (H^{+}). Originally biomining has been applied in extracting valuable desirable metals (e.g. copper) from low-grade ores (Schippers et al. 2014; Brierley and Brierley, 2013; Gahan et al., 2012). However, biomining procedures can be carried out for metal extraction from tailings and other mine wastes or as *in situ* leaching with obvious cost advantages and minimal disturbance to the surrounding environment (Schippers et al., 2014).

Biomining includes bioleaching and biooxidation processes. Both processes are oxidation processes, but in biooxidation the valuable metals are left more enriched in the solid phase while in bioleaching the valuable metals are released into aqueous phase (Figure 2). Biooxidation is often used, for instance, in the pre-treatment of gold concentrates, prior to conventional cyanide-extraction while bioleaching is used for the recovery of metals (e.g. copper) from low-grade ores (Schippers et al., 2014; Brierley and Brierley, 2013; Gahan et al., 2012).

Today bioleaching is being applied as the main process in large-scale operations (Acevedo, 2000). It takes place in highly aerated continuous-flow/stirred-tank reactors or in irrigated dump/heap reactors (open and non-sterile environments) or *in-situ* leaching in underground mines/ ore deposits. Dump/heap reactors provide highly heterogeneous growth environments that change with the age of the heap and variety

of acidophilic ISOPs are involved in the processes (Schippers et al., 2010; Remonsellez et al., 2009; Demergasso et al., 2005).

In contrast, stirred-tank reactors are characterized by homogeneous and constant growth conditions and two or three species of acidophilic ISOPs can be dominant (Rawlings and Johnson, 2007).

Nowadays, the importance of biomining technology is clearly recognized due to the increasing world demand for some valuable metals such as copper (Schippers et al., 2014; Acevedo, 2002). Due to increasing research in this field, the number of reported genera and species of acidophilic ISOPs involved in the oxidation of metal sulfides has increased substantially since the 1970s. Many researchers are investigating the microorganisms and their fundamental characteristics in different environments such as hot springs, volcanic regions, and mining operations that support the growth and activities of these organisms.

The studies provided mesophilic, moderate thermophilic and thermophilic acidophilic ISOPs for biomining processes (Vera et al., 2013; Schippers, 2007). Due to some research papers, acidophilic thermophilic archaea can be more effective than mesophilic bacteria in the oxidation of primary copper sulfides such as chalcopyrite (Ding et al., 2011; Jarrell et al., 2011; Konishi et al., 1995; Larrson et al., 1990).

Acidophilic ISOPs can oxidize and leach the metal sulfides in two different modes: ‘‘contact’’ and ‘‘non-contact’’ leaching. ‘‘Contact’’ and ‘‘non-contact’’ leaching have replaced the formerly often used terms “direct” and “indirect” leaching. Non-contact leaching is basically done by planktonic bacteria, whereas contact leaching refers to cells attached to the surface of metal sulfide minerals. In contact leaching, electrochemical processes that result in the dissolution of sulfide minerals, take place at the interface between the bacterial cell and the mineral sulfide surface. This space

is filled with extracellular polymeric substances (EPS) where likely high amounts of ferric iron serve as efficient oxidant. However in both modes “non-contact” and “contact” leaching, metal sulfide dissolution by acidophilic ISOPs results in the generating of ferric iron and sulfuric acid (Vera et al., 2013; Schippers and Sand, 1999).

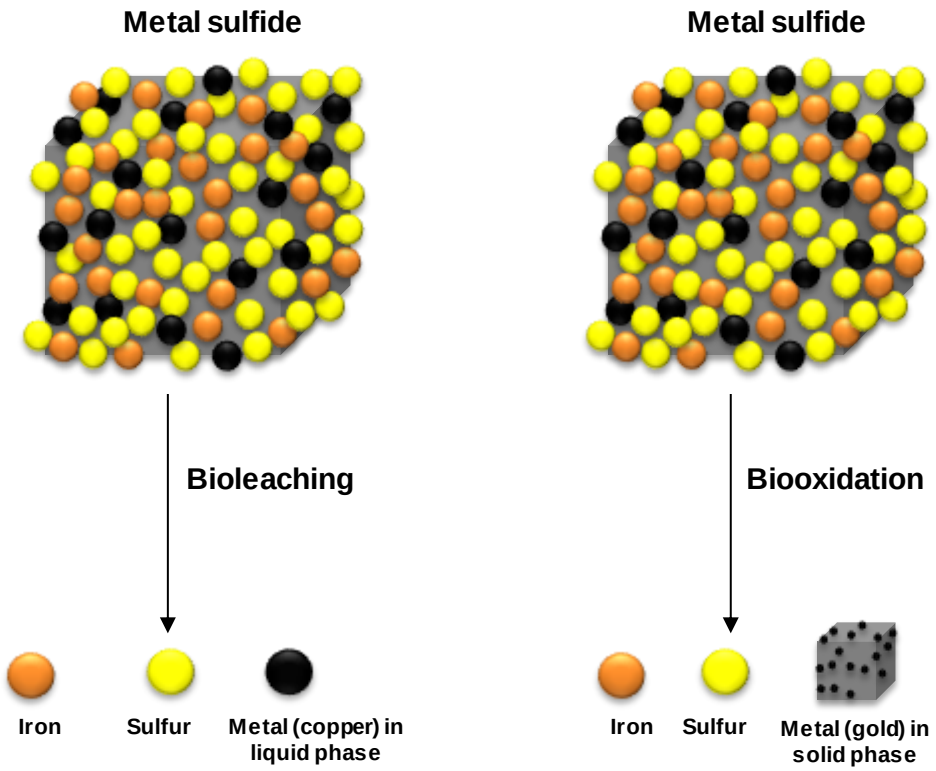

Figure 2. Scheme of bioleaching versus biooxidation. In the metal sulfide, sulfur (yellow) is bound to iron (orange and/or other metals (black) such as e.g. copper or zinc. Gold occurs in the mineral matrix. Iron and sulfur are oxidized by microbial activity, and in case of bioleaching, metals are leached into solution, while during biooxidation the desired metal (gold) remains enriched and accessible in the solid phase.

From the chemical point of view, metal sulfide oxidation can occur through two different pathways/mechanisms which were identified as thiosulfate and polysulfide pathways/mechanisms (Figure 3) according to the mineralogical properties of the

metal sulfides as well as the geochemical conditions (Schippers et al., 2014; Schippers and Sand, 1999).

The terms thiosulfate and polysulfide pathway refer to the occurrence of the intermediate sulfur compounds during oxidation of two different groups of metal sulfides; acid-insoluble metal sulfides and acid-soluble metal sulfides (most metal sulfides). Acid-insoluble metal sulfides such as pyrite (FeS_2) can be only oxidized by chemical attack of ferric iron (Fe^{3+}).

Due to attack of ferric iron, the sulfur moiety is oxidized to thiosulfate as the first soluble sulfur intermediate. Subsequently, thiosulfate is oxidized to tetrathionate which can be further degrading to various sulfur compounds such as trithionate, pentathionate, elemental sulfur and sulfite. All sulfur compounds which are by-product of the thiosulfate mechanism are finally oxidized to sulfate and protons as summarized in equations 6 and 7.

$$FeS_2 + 6Fe^{3+} + 3H_2O \longrightarrow S_2O_3^{2-} + 7\,Fe^{2+} + 6H^+ \quad \text{(eq. 6)}$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \longrightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+ \quad \text{(eq. 7)}$$

Other metal sulfides such as chalcocite as one of the most important copper mineral are acid-soluble and are degraded by a combined attack of protons and ferric iron. The main intermediates of this process are polysulfides (S_n^{2-}) and elemental sulfur (S^0) which can be further oxidized to sulfate and protons (Equation 8-10).

$$Cu_2S + 3\,Fe^{3+} + H^+ \longrightarrow 2\,Cu^{2+} + 0.5\,H_2Sn + 3\,Fe^{2+}\ (n>2) \quad \text{(eq. 8)}$$

$$0.5\,H_2S_n + Fe^{3+} \longrightarrow 0.125\,S_8 + Fe^{2+} + H^+ \quad \text{(eq. 9)}$$

$$0.125\,S_8 + 1.5\,O_2 + H_2O \longrightarrow SO_4^{2-} + 2\,H^+ \quad \text{(eq.10)}$$

Table 2. Metal sulfides oxidation ability of acidophilic ISOPs. na= data not available (from Schippers et al., 2014, modified).

Species	Oxidation of	
	Pyrite	Other MS
Mesophiles		
Acidiferrobacter thiooxydans	+	na
Acidithiobacillus ferrooxidas	+	+
Acidithiobacillus thiooxidans	-	+
Acidithiobacillus ferrivorans	+	+
Acidithiobacillus ferridurans	+	+
Acidithiobacillus albertensis	-	+
‘‘*Thiobacillus prosperus*’’	+	+
‘‘*Thiobacillus plumbophilus*’’	-	+
Thiomonas cuprina	-	+
Leptospirillum ferrooxidans	+	+
Leptospirillum ferriphilum	+	+
‘‘*Leptospirillum ferrodiazotrophum*’’	na	na
Ferrimicrobium acidiphilum	+	na
Alicyclobacillus disulfidooxidans	+	na
Alicyclobacillus tolerans	+	+
Sulfobacillus benefaciens	+	na
‘‘*Sulfobacillus montserratensis*’’	+	na
Sulfobacillus thermotolerans	+	+
‘‘*Ferroplasma acidarmanus*’’	+	na
Ferroplasma acidiphilum	+	na
Moderate thermophiles		
Acidithiobacillus caldus	-	+
Acidimicrobium ferrooxidans	+	na
“Acidithiomicrobium P1/P2”	na	+
Ferrithrix thermotolerans	+	na
Sulfobacillus acidophilus	+	+
Sulfobacillus sibiricus	+	+
Sulfobacillus thermosulfidooxidans	+	+
Acidiplasma cupricumulans	na	+
Thermophiles		
Acidianus brierleyi	+	+
Acidianus infernus	+	+
Acidianus sulfidivorans	+	+
Metallosphaera hakonensis	na	+
Metallosphaera prunae	+	+
Metallosphaera sedula	+	+
Sulfolobus metallicus	+	+
Sulfolobus yangmingensis	na	+
Sulfurococcus yellowstonensis	+	+
Sulfurococcus mirabilis	+	+

Acidophilic ISOPs are responsible for ferrous iron and sulfur oxidation (equation 1-5) in both the thiosulfate and the polysulfide mechanisms (Schippers et al., 2014; Schippers and Sand, 1999).

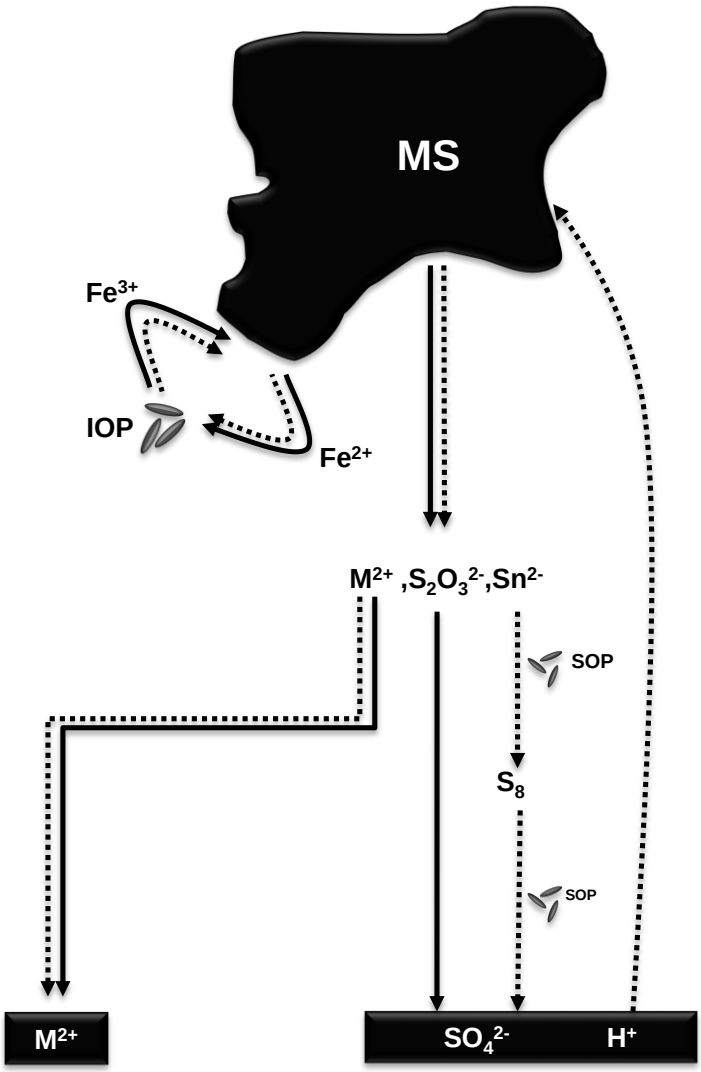

Figure 3. A schematic of the two metal sulfide oxidation pathways. The solid line and short-dashed lines represent thiosulfate and polysulfide mechanisms/pathways, respectively. MS= metal sulfide, IOMs= iron-oxidizing porkaryotes and SOPs= sulfur-oxidizing prokaryotes (modified from Sand and Schippers, 1999).

The explained chemical processes (equations 1-10) which are mediated by acidophilic ISOPs can occur in all metal sulfidic systems irrespective of whether tank or dump reactors are used and/or natural processes occur in mine tailings or acid mine/rock drainage (AMD/ARD) environments.

1.3.2. Environmental perspective: sulfidic mine tailings and acid mine/rock drainage (AMD/ARD)

After a successful exploration of an ore body, ores are extracted by mining (open pit or underground mine) and then transported to stockpiles or directly processed. During milling process the ores are crushed and milled to reduce the grain size. Subsequently, the fine grains are mixed with water and chemical reagents to separate economical important minerals from waste minerals during the flotation process. After that the residual material which is termed tailings is transported in the form of suspension to impoundments for final deposition (Figure 4). In the case of porphyry copper mines in Chile, typically 95-99% of the processed ore are deposited as tailings which contain 0.4-4% sulfur, mainly as pyrite (FeS_2) (Dold, 2010; Diaby et al., 2007). Once tailings deposition cease, water levels in tailings impoundments fall down (in non-management tailings case) and a water unsaturated zone become exposed to atmospheric oxygen. Consequently, tailings undergo oxidation and an oxidized zone is formed above the saturated, not oxidized zone (Dold, 2007). In the oxidation zone where oxidation actually takes place, metal sulfides, which mainly include pyrite, pyrrhotite and chalcopyrite in the case of porphyry copper mines, are dissolved by iron and sulfur oxidation processes. At pH values above 4, the oxidation of iron is chemical by oxygen while below pH 4 the chemical oxidation of iron is negligible and acidophilic ISOPs mediate the oxidation processes. Acidophilic ISOPs can accelerate metal sulfide (e.g. pyrite) oxidation reaction rate 30-300fold (Nordstrom and Alpers, 2000) and therefore high amounts of metalloids and heavy metals can be released to the environment (Schippers et al., 2010; Diaby et al., 2007). Released metals can be mobilized, under acidic condition, in response to climate condition either towards the

surface of a tailings dump (in arid climate) or towards the groundwaters (humid climate).

In both cases, the acidic, metal-rich solution (AMD/ARD) can be released from the tailings and contaminate the environment (Anawar, 2013; Schippers et al., 2010; Rohwerder and Sand, 2007; Akcil and Koldas, 2006; Kappler and Newman, 2004; Johnson, 1998). AMD/ARD is considered as one of the most serious contamination source for environments and their inhabitants throughout the world. Rio Tinto in Spain, Iron Mountain in California, USA with partly an extremely low pH of below zero, the tailings dumps in Europe, western India, South America (Chile and Peru), South Africa and Australia are examples of AMD/ARD sites in different climate zones (Schippers et al., 2010; Mendez et al., 2008).

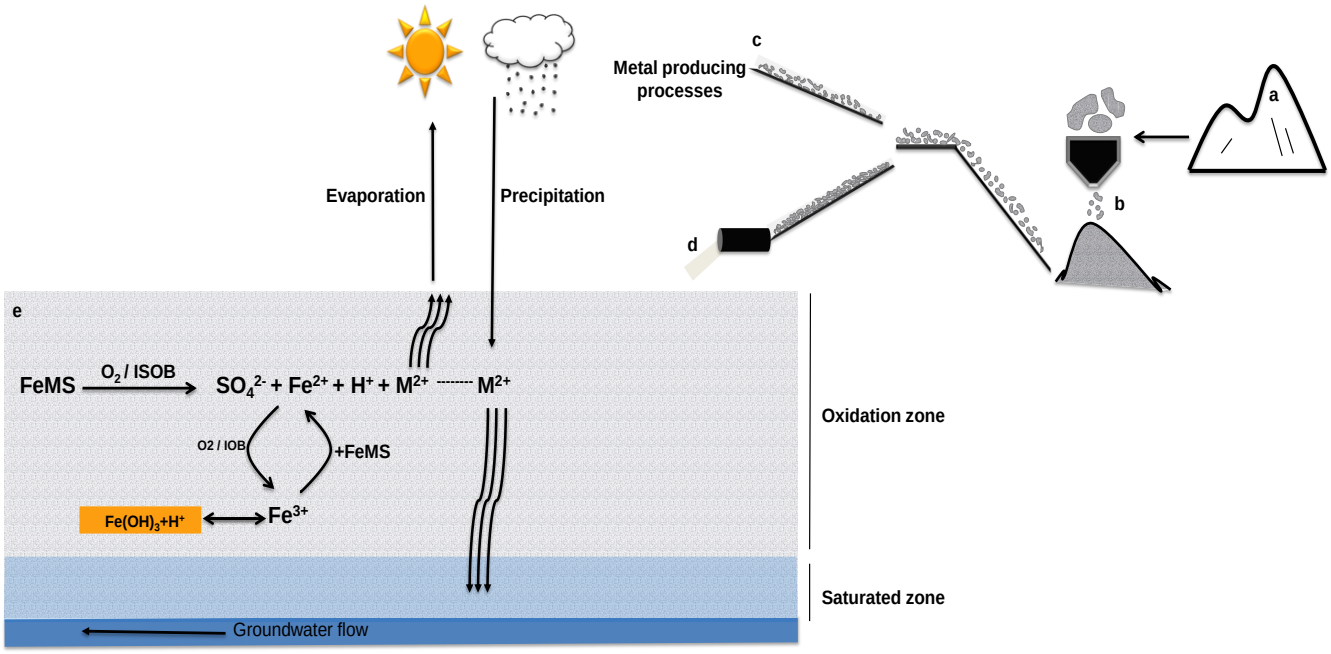

Figure 4. A schematic of tailings production, deposition and oxidation processes in a mine. a= mine, b= ore crushing, c= metal producing processes including milling and pyrometallurgy or hydrometallurgy, d= deposition of tailings in suspension, e= oxidation processes in mine tailings dump.

Various geochemical factors such as type and amount of metal sulfide, pH, temperature, oxygen content, chemical state of iron and microbial community are

affecting AMD/ARD generation (Akcil and Koldas, 2006). Although low pH and high conductivity are the traits of all AMD/ARD throughout the world, the geochemical and microbial compositions of them varies due to differentiations of geological and mineralogical properties and climate of each tailings dump. Since sulfidic mine tailings are poor in carbon and nitrogen, acidophilic ISOPs which can obtain their required energy chemolithotrophically and are able to fix nitrogen, dominate the microbial communities of tailings dumps (Schippers et al., 2010; Kock and Schippers, 2008; Akcil and Koldas, 2006).

The most important acidophilic ISOPs which can be found in tailings dumps belong to the genus *Acidithiobacillus* such as *At. ferrooxidans* or *At. thiooxidans*. *At. ferrooxidans* is dominant in many biomining processes and tailings systems due to its ability to grow with ferrous iron (Fe^{2+}), various sulfur compounds and consequently pyrite (FeS_2), chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), covellite (CuS) and so on (Schippers et al., 2014; Schippers, 2007). *At. thiooxidans* is not able to degrade sulfide minerals such as pyrite efficiently (Table 2) due to lack of ferrous iron oxidation ability but it can remove the sulfur layers which might be forming on the surfaces of minerals and allow IOMs to further oxidize the metal sulfides and in consequence accelerate the dissolution rate of metal sulfides. In addition to Gram-negative ISOPs, metal sulfide oxidizing Gram-positive bacteria which belong to e.g. the acidophilic ferrous iron and sulfur compound-oxidizing genera *Alicyclobacillus* and *Sulfobacillus* are also found in sulfidic mine tailings (Schippers et al., 2010; Diaby et al., 2007). Besides bacteria, acidophilic archaea such as *Ferroplasma acidiphilum* could be determined in tailings dumps (Huang et al., 2011; Tan et al., 2008).

1.4. Molecular methods to analyze microbial communities

Since different physiological groups of bacteria and archaea are responsible for metal sulfide oxidation, identification and quantification of single species in complex bioleaching communities supports the selection of the relevant strategy to control and optimize metal bioleaching as well as to minimize their impacts on for instance, acid mine/rock drainage (AMD/ARD) generation. In general, culture-dependent and culture-independent methods can be utilized to analyse microbial communities. Utilizing both methods can give a more complete picture of microbial communities in an environment (Auld et al., 2013). Culture-independent methods are quick and reliable methods to determine phylogenetic composition and structure of the microbial community (Schippers, 2007; Tan et al., 2007). Nucleic-acid based molecular approaches are based on the extraction of DNA or RNA from a culture, a bioreactor or an environmental sample. In most cases, the 16S ribosomal RNA gene (16S rRNA gene) of prokaryotes (*Bacteria* and *Archaea*) is targeted, but also functional genes coding for key enzymes of particular metabolic interest have been analysed (e.g. the *rus* gene coding for rusticyanin in *At. ferrooxidans*). In case of identification of microorganisms to address biodiversity or a new species, DNA is extracted and the 16S rRNA gene is amplified using the polymerase chain reaction (PCR). The gene sequence is determined after isolation in clone libraries. The identification of the similarities of the sequences with those in data bases allows for a determination of the phylogenetic affiliation of the microorganisms in the sample. Further, the phylogenetic affiliation can be shown as a phylogenetic tree.

Besides identification of microorganisms phylogenetically, real-time quantitative PCR (qPCR), fluorescence in situ hybridization (FISH) or its modification catalyzed reporter deposition – fluorescence in situ hybridization (CARD – FISH) are used for identifying

of microbial communities quantitatively and qualitatively (Schippers and Bosecker, 2005; Pernthaler et al., 2002). In environmental microbiology qPCR is a technique with high sensitivity and often used to quantify different phylogenetic groups and genera. The technique is based on the online fluorescence detection of PCR products and allows the rapid detection and quantification of gene sequences without the need for labour-intensive post-PCR processing (Bowei et al., 2009). There are different chemical assays for real-time PCR, but the most common are sequence-specific TaqMan probes and the intercalating non-specific SYBR-Green dye which allows theoretically the detection of a single DNA molecule. The detection limit of the method depends on the target of interest, sample purity, PCR conditions and other factors. Furthermore, SYBR-Green based approaches have been developed to quantify single species in bioleaching communities such as *Acidianus brierleyi*, *Sulfobacillus thermosulfidooxidans*, *Acidithiobacillus caldus*, and *Leptospirillum ferrooxidans* (Liu et al., 2006).

Another powerful technique to quantify microbial cells in environmental samples is FISH. Since FISH targets ribosomal RNA (rRNA), which is indicative of actively metabolizing bacteria, FISH can provide quantitative information on living bacteria in an environmental sample. FISH has been successfully applied to quantify acidophilic Fe(II)-oxidizing *Acidithiobacillus*, *Leptospirillum*, *Ferroplasma* and other microorganisms in acid mine drainage environments and in bioleaching operations. A drawback of the technique is that a sufficient content of cellular ribosomes is prerequisite for its successful application (Schippers, 2007). The modified FISH method is CARD-FISH which allows the detection of less active cells in environmental samples (Pernthaler et al., 2002) such as in mine tailings (Kock and Schippers, 2008).

1.5. Aims of work

This work addresses three different tasks (Chapter 2). First, possible life and biogeochemical processes in a multiple extreme, high-saline, metal-rich, acidic and almost organic carbon-free copper mine tailings dump in arid climate of the Atacama Desert at Chañaral Bay, northern Chile have been explored via an application of various cultivation-dependent and independent techniques. The microbial community as well as geochemical and mineralogical properties of 50 marine shore mine tailings samples from four sites were analysed qualitatively and quantitatively. Considering also a potential application in biomining, acidophilic halotolerant iron-oxidizing microorganisms were enriched at salt concentrations of up to 1 M and their capability to oxidize iron(II) at high salinity was investigated (Chapter 3).

The second task was to investigate the composition of microbial communities in moderate acidic mine tailings in comparison to those in extreme acidic mine tailings mainly described in the literature. Therefore, the microbial diversity was analyzed via 16S rRNA gene sequencing in eight selected samples from three different mine tailings dumps in different climate zones: humid and cold (Kristineberg in Sweden), humid and temperate (Freiberg in Germany) and warm and semi-arid (Selebi-Phikwe in Botswana) (Chapter 4).

The third task was to test a comparative dissolution of chalcopyrite in a marine hydrothermal massiv sulfide ore via bioleaching at different temperatures. Chalcopyrite is the main copper-bearing mineral of the world and is often remaining in tailings and after heap leaching operations. In laboratory batch experiments, bioleaching of the ore by mesophilic, moderate thermophilic or thermophilic acidophilic iron- and sulfur-oxidizing prokaryotes was examined (Chapter 5).

Chapter 2

Results and discussion

2. Results and discussion

This chapter summarises the results of this work presented in three manuscripts (chapters 3-5) and includes brief overall discussions.

2.1. Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile

Overall, geochemical conditions are very different among different tailings sites and depths, and may have significant influence on the microbial community composition. Fluctuation of dominant species within such inhomogeneous layering tailings horizons is very complex and poorly understood (Kock and Schippers, 2008).

Figure 5. Secondary copper minerals on the surface of the tailings dump at Chañaral Bay and on the marine shells (Picture from BGR).

The copper tailings dump at Chañaral Bay is characterized by high acidity, high salinity and high heavy metals concentration. Due to high evaporation, the released metals from pyrite oxidation processes are mobilized under acidic conditions towards the surface of the tailings dump and precipitate as secondary minerals such as chlorides and sulfates (Figure 5). Acidophilic chemolithotrophic iron- and sulfur-oxidizing bacteria are an important group of microorganisms present in this extreme environment and involved in biogeochemical processes such as metal and sulfur cycling. The highest total cell number of microorganisms determined by SYBR-Green direct counting (SGDC) was up to 10^8 cells/g dry weight. The CARD-FISH analysis gave somewhat lower counts which shows that a high proportion of detectable microorganisms is alive. Also, the cultivation based most-probable-number quantification of acidophilic iron-

oxidizers detected living bacteria but numbers of living cells did not exceed 10^3 cells/g dry weight which is low in comparison to other less extreme tailings where up to 10^9 cells/g dry weight were found with the same method (Kock and Schippers, 2008). Depth profiles of the abundance of iron-oxidizers coincided with biological pyrite oxidation rates measured via microcalorimetry as well as the pyrite content. Due to this finding and the extremely low organic carbon content in the tailings, metal sulfides are considered to be the main energy source for microorganisms in the tailings dump at Chañaral Bay. The cell numbers of *Bacteria* detected by q-PCR were in the same magnitude or somewhat lower than total cell counts. *Archaea* were less abundant than Bacteria supported by CARD-FISH results which emphasized the role of *Bacteria* in biogeochemical processes in tailings in agreement with previous studies abot tailings microbiology (Schippers et al., 2010; Kock and Schippers, 2006).

Specific qPCR assays detected the acidophilic iron- and sulfur-oxidizers *Acidithiobacillus* spp., and, *Sulfobacillus* spp., as well as anaerobic sulfate reducers via their *dsr*A functional gene in the tailings dump at Chañaral Bay. *Acidithiobacillus* spp. was the most abundant iron- and/or sulfur-oxidizer. The sulfur-oxidizer *Acidithiobacillus caldus* was found only in few samples with the highest number of 10^4 cells/g dry weight. The obligate acidophilic iron-oxidizer *Leptospirillum* spp. was not found via qPCR at Chañaral Bay (Chile) and also not in Selebi-Phikwe (Botswana) where the mean sodium concentration was above 1% wt (Korehi et al., 2013; Schippers et al., 2007). In soil covered pyrite-containing tailings in Kristineberg (Sweden), *Leptospirillum* spp. occurred in numbers similar to *Acidithiobacillus* spp. and was also identified in low numbers in a tailings dump in uncovered pyrite and arsenopyrite-containing tailings dump in Freiberg (Germany) (Kock and Schippers, 2008).

The bacterial phylogenetic survey via 16S rRNA gene sequencing showed the occurrence of the acidophilic iron- and sulfur-oxidizing and iron-reducing *Acidithiobacillus*, *Alicyclobacillus*, and *Sulfobacillus* in the tailings dump at Chañaral Bay. In addition, the 16S rRNA gene sequences showed the presence of relatives of chemolithoautotrophic sulfur-oxidizing *Thiomicrospira frisia* (Brinkhoff et al.,1999) and *Th. crunogena* (Scott et al., 2006; Niederberger et al., 2009; Wirsen et al., 1998), and moderately halophilic, obligately chemolithoautotrophic, sulfur-oxidizing *Thiohalomonas denitrificans* (Sorokin et al., 2007) in Chañaral Bay at the marine shoreline (site CH12).

In addition to the microbial community analysis, for the first time, the iron-oxidizing ability of acidophilic ISOPs at high salt concentration of 1 M NaCl has been determined. This new finding gives a perspective to perform biomining with seawater in arid area or area with fresh water shortage (Watling, 2014; Kamimura et al., 2001). The 16S rRNA gene sequencing showed an affiliation of the halotolerant bacteria in our stable, most active enrichment culture to the iron- and sulfur-oxidizer *Sulfobacillus* spp.

2.2. Microbial diversity at the moderate acidic stage in three different sulfidic mine tailings dumps generating acid mine drainage

Mine tailings dumps are an extreme environment which is characterized by low microbial diversity in comparison to other environments (Korehi et al., 2013; Chen at al., 2013; Mendez et al., 2008). Geochemical conditions determine the microbial community composition, and most relevant for mine tailings seems to be the pH. In contrast to tailings at pH<3, the moderate acidic stage in mine tailings is only scarcely studied. Thus, the microbial communities of three different sulfidic and acidic mine

waste tailing dumps in Selebi-Phikwe, Botswana (semi-arid, warm), Freiberg, Germany (humid, temperate) and Kristineberg, Sweden (humid, cold) at the pH range of 3.2 to 6.5 were analyzed by 16S rRNA gene diversity analysis via clone libraries. The 16S rRNA gene sequencing analysis of tailings dumps samples showed the dominance of *Proteobacteria* and *Firmicutes*. Sequences of near-surface clones from Selebi-Phikwe showed that the bacterial population belonged to various phylogenetic groups, representing *Proteobacteria* (47%), *Firmicutes* (44%), *Actinobacteria* (8%) and *Nitrospirae* (1%). Also, most abundant phylotypes in the clone libraries were closely related to *Alicyclobacillaceae* within the *Firmicutes* and *Hydrogenophilaceae* within *Proteobacteria*. Sequencing of clones from the subsurface up to 11 m depth showed the dominance of *Firmicutes* while the most abundant phylotype was closely related to *Peptococcaceae*. This family comprises anaerobic, sulfate-reducing bacteria (Rogosa, 1971), previously found in other mine tailings (Schippers et al., 2010; Fortin et al., 1996).

The same tendency was observed in the microbial community changes with depth in Kristineberg. *Firmicutes* were most abundant in deeper layers while 16S rRNA gene sequences at the surface were mainly related to *Proteobacteria* (49%) and *Actinobacteria* (47%). Within *Firmicutes* the families *Alicyclobacillaceae* and *Peptococcaceae* were highly abundant in the clone libraries. *Proteobacteria* were also the dominant phylum in Freiberg, but, in contrast to the Selebi-Phikwe and Kristineberg samples, the phylum *Firmicutes* was not identified in the microbial community at Freiberg. In agreement with the data of this thesis, previous studies also revealed members of the phyla *Proteobacteria*, *Firmicutes* and *Actinobacteria* as major microbial groups in mine tailings. Besides representatives of other phyla which were identified in the communities of the three different tailings dumps studied in this thesis,

have also been found in heavy-metal contaminated soils and tailings dumps as moderate or extremely acidophilic heavy metal resistant microorganisms (Giloteaux et al.,2013; Barns et al., 2012; Huang et al., 2011; Mendez et al.,2008). Overall, the results revealed the differentiation of the microbial diversity between three different mine tailings dumps and related to metal sulfide oxidation processes due to pH changes. Accordingly, the microbial community compositions was different in these moderate acidic tailings dumps in comparison to acidic tailings dumps in which acidophilic iron- and sulfur-oxidizing bacteria prevail (Schippers et al., 2010).

2.3. Bioleaching of a marine hydrothermal sulfide ore with mesophiles, moderate thermophiles and thermophiles

Chalcopyrite ($CuFeS_2$) is the main copper-bearing mineral and recovered from terrestrial ores by mining and ore processing. Since bioleaching of chalcopyrite is still quite inefficient at ambient temperature, a lot of research is undertaken to improve chalcopyrite bioleaching. The oxidation of chalcopyrite by acidophilic iron- and sulfur-oxidizing prokaryotes provides protons and ferric iron (Fe^{3+}) (Chapter 1; Equation 9-10) that are the main agents of chalcopyrite oxidation (Dorado et al., 2012; Rodriguez et al., 2003; Sand and Schippers, 1999). Although the rate of chalcopyrite dissolution is increased at strongly acidic conditions and at high concentrations of ferric iron, other factors such as temperature also affect the chalcopyrite dissolution kinetics (Koleini et al., 2010;

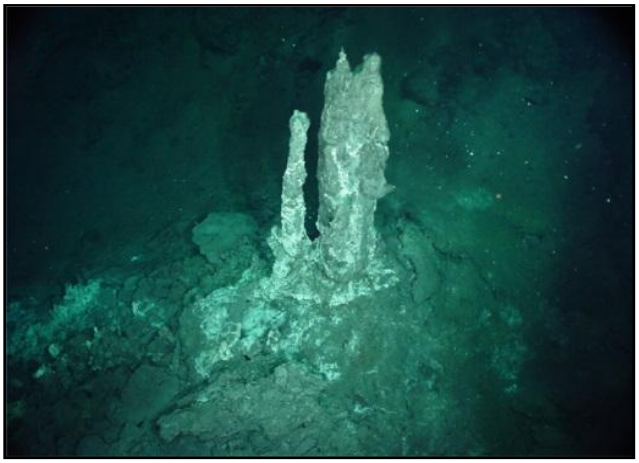

Figure 6. Inactive sulfide chimney in the Indian Ocean (Picture from BGR).

Cordoba et al., 2008; Mousavi et al., 2005; Nemati and Harrison, 2000). Bioleaching of chalcopyrite from terrestrial ore was shown to be more efficient at high temperature using thermophilic iron- and sulfur-oxidizing archaea than mesophilic bacteria (Konishi et al. 2001; Konishi et al., 1999). Marine hydrothermal polymetallic sulfide ores contain high amounts of valuable metals including copper as chalcopyrite. In order to test if chalcopyrite bioleaching takes place in a similar manner than described for terrestrial chalcopyrite, bioleaching experiments with a marine hydrothermal sulfide ore from the Indian Ocean (Figure 6) were carried out at different temperatures over a period of 4 weeks. The experimental results showed that the copper leaching rate at 2% (w/v) pulp density of chalcopyrite was higher with the thermophilic archaea (*Acidianus brierleyi*) than with moderate thermophilic and mesophilic bacteria. The first step in all bioleaching cultures was initial ferrous iron oxidation and increasing initial pH and redox-potential. During this short initial step (within the first week) more copper was leached to solution by moderate thermophilic than by thermophilic and mesophilic microorganisms probably because of the best adaptation to the mineral of the mixed culture of moderate thermophiles. But finally, maximal copper dissolution was observed for the culture of thermophilic archaea at a redox potential close to 440 mV (vs. Ag/AgCl) that was less than that for the mesophilic and moderate thermophilic cultures with values of 550 to 600 mV. Sandström et al. (2005) showed that chalcopyrite leaches more readily at lower redox potential. The results showed that an increase in temperature from 30°C to 70°C had a major impact on the bioleaching efficiency since the copper and iron extraction efficiency occurred in the order thermophiles, moderate thermophiles, and mesophiles. The chalcopyrite leaching rate was maximal in the presence of the thermophilic archaea *A. brierleyi*, in agreement with data for bioleaching of chalcopyrite from terrestrial ore (Konishi et al. 2001;

Konishi et al. 1999). These findings indicate that chalcopyrite bioleaching is controlled by different factors including redox potential and temperature and that bioleaching of chalcopyrite from a marine ore is not different to that from terrestrial ores.

References

1. Acevedo F, Present and future of bioleaching in developing countries, EJB Electronic Journal of Biotechnology, 5 (2) (2002), 196-199.
2. Acevedo F, The use of reactors in biomining processes, EJB Electronic Journal of Biotechnology, 3 (3) (2000), 184-194.
3. Akcil A, Koldas S, Acid Mine Drainage (AMD): causes, treatment and case studies, Journal of Cleaner Production, 14 (2006), 1139-1145.
4. Alam M, Pyne P, Manzumdar A, Peketi A, Ghosh W, Kinetic enrichment ^{34}S during proteobacterial thiosulfate oxidation and the conserved role of SoxB in S/S bond breaking, Appl. Environ. Microbiol., 79 (14) (2013), 4455–4464.
5. Anawar H. M, Impact of climate change on acid mine drainage generation and contaminant transport in water ecosystems of semi-arid and arid mining areas, Physics and Chemistry of the Earth, 58–60 (2013), 13–21.
6. Auld R. R, Myre M, Mykytczuk N. C.S, Leduc L.G, Merritt T. J.S, Characterization of the microbial acid mine drainage microbial community using culturing and direct sequencing techniques, J. Microbiol. Meth., 93 (2013), 108–115.
7. Barns S. m, Cain E. C, Sommerville L, Kuske C. R, Acidobacteria phzlum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum, 73 (9) (2007), 3113-3116.
8. Bauer M, Richter M, Würdemann C. A, Kuhl H, Bischof K, Kube M, Lombardot T, Teeling H, Quast C, Knaust F, Allers E, Woebken D, Mussmann M, Choudhuri J. V, Reinhardt R, Meyer F, Amann R. I, Glöckner F. O, Whole genome analysis of the marine *Bacteroidetes Gramella forsetii* reveals adaptations to degradation of polymeric organic matter, Environ. Microbiol., 8 (12) (2006), 2201-2213.
9. Benner S. G, Gould W.D, Blowes D. W, Microbial populations associated with the generation and treatment of acid mine drainage, Chem. Geol. 169 (2000), 435-448.
10. Bonnefoy V, Holmes D. S, Genomic insights into microbial iron oxidation and iron uptake strategies in extremely acidic environments, Environ. Microbiol., (2011), 1-15.
11. Bowei C, Xingyu L, Wenyan L, Application of clone library analysis and real-time PCR for comparison of microbial communities in a low-grade copper sulfide ore bioheap leachate, J. Ind. Microbiol. Biotechnol., 36 (2009), 1409–1416.
12. Brierley C. L, Brierley J. A, Progress in bioleaching: part B: applications of microbial processes by the minerals industries, Appl. Environ. Microbiol., 97 (2013), 7543-7552.

13. Brinkhoff T, Sievert S. M, Kuever J, Muzyer G, Distribution and diversity of sulfur-oxidizing *Thiomicrospira* spp. at a shallow-water hydrothermal vent in the Aegean Sea (Milos, Greece), Appl. Environ. Microbiol., 65 (9) (1999), 3843-3849.
14. Brock T. D, Brock K. M, Bellz R. T, Weiss R. L, *Sulfolobus*: A new genus of sulfur-oxidizing bacteria living at low pH and high temperature, Arch. Microbiol., 84 (1972), 54-68.
15. Chen L, Ren Y, Lin J, Liu X, Pang X, Lin J, *Acidithiobacillus caldus* Sulfur Oxidation Model Based on comb. nov.: Facultatively Aerobic, Extremely Acidophilic Thermophilic Sulfur-Metabolizing Archaebacteria, Int. J. Syst. Bacteriol., 36 (4) (1986), 559-564.
16. Chen L. X, Li J. T, Chen Z. T, Huang L. N, Hua Z. S, Hu M, Shu W. S, Shifts in microbial community composition and function in the acidification of a lead/zinc mine tailings, Environ. Microbiol.,15 (9) (2013), 2431-2444.
17. Cleaver A. A, Burton N. P, Norris P. R, A Novel *Acidimicrobium* species in continuous cultures of moderately thermophilic, mineral-sulfide-oxidizing acidophiles, Appl. Environ. Microbiol. 73 (13) (2007), 4294-4299.
18. Córdoba E.M, Muñoz J.A, Blázquez M.L, González F, Ballester A, Leaching of chalcopyrite with ferric ion. Part II: Effect of redox potential, Hydrometallurgy 93 (2008), 88-96.
19. De Rosa M., Gambacorta A., Bulock J. D., Extremely thermophilic acidophilic bacteria convergent with *Sulfolobus acidocaldarius*, J. general. Microbiol. 86 (1975), 156-164.
20. Demergasso C. S, Galleguillos P. A, Escudero L. V, Zepeda V. J, Castillo D, Casamayor E. O, Molecular characterization of microbial populations in a low-grade copper ore bioleaching test heap, Hydrometallurgy, 80 (2005), 241-253.
21. Diaby N, Dold B, Pfeifer H. R, Holliger C, Johnson D. B, Hallberg K. B, Microbial communities in a porphyry copper tailings impoundment and their impact on the geochemical dynamics of the mine waste, Environ. Microbiol., 9 (2) (2007), 298-307.
22. Ding J, Zhang R, Yu Y, Jin D, Liang C, Yi Y, Zhu W, Xia J, A novel acidophilic, thermophilic iron and sulfur-oxidizing archaeon isolated from a hot spring of Tengchong, Yunnan, China, Braz. J. Microbiol., 42 (2011), 514-525.
23. Dold B, Basic Concepts in Environmental Geochemistry of Sulfidic Mine-Waste Management. In: Kumar ES (Ed.): Waste Manage., In Tech, (2010), 173-198.
24. Dold B, Biogeochemical processes in mine tailings with special focus on marine shore tailings deposits and their remediation, Adv. Mat. Res., 20-21 (2007), 177-185.
25. Dopson M, Johnson D. B, Biodiversity, metabolism and application of acidophilic sulfur-metabolizing microorganisms, Environ. Microbiol., 14 (10) (2012), 2620-2631.
26. Dorado A.D, Solé M, Lao C, Alonso P, Gamisans X, Effect of pH and ferric ions on chalcopyrite bioleaching by an adapted consortium from biogas sweetening, Miner. Eng., 39 (2012), 1-18.
27. Edwards K. J, Gihring T.M, Banfield J. F, Seasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment, Appl. Environ. Microbiol., 65 (8) (1999), 3627-3632.
28. Emerson D, Fleming E. J, Mc Beth J. M, Iron-oxidizing bacteria: an environmental and genomic perspective, Annu. Rev. Microbiol., 64 (2010), 561–83.

29. Friedrich C.G, Rother D, Bardischewsky F, Quentmeier A, Fischer J, Oxidation of reduced inorganic sulfur compounds by Bacteria: emergence of a common mechanism?, Appl. Environ. Microbiol., 67 (7) (2001), 2873-2882.
30. Fortin D, Davis B, Beveridge T. J, Role of *Thiobacillus* and sulfate-reducing bacteria in iron biocycling in oxic and acidic mine tailings, FEMS Microbiol. Ecol., 21 (1996), 11-24.
31. Gadd G. M, Raven J. A, Geomicrobiology of Eukaryotic Microorganisms, Geomicrobiol. J., 27 (2010), 491–519.
32. Gahan C. S, Sundkvist J. E, Dopson M, Sandström, Effect of chloride on ferrous iron oxidation by a *Leptosprillum ferriphilum*-dominated chemostat culture. Biotechnol. Bioeng., 106 (3) (2010), 422-431.
33. Gahan C.S, Srichandan H, kim D. J, Akcil A, Biohydrometallurgy and biomineral processing technology: a review on its past, present and future. Res. J. Recent Sciences, 1 (10) (2012), 85-99.
34. Giloteaux L, Holmes D. E, Williams K. H, Wrighton K. C, Wilkins M. J, Montgomery A. P, Smith J. A, Orellana R, Thompson C. A, Roper T. J, Long P. E, Lovley D. R, Characterization and transcription of arsenic respiration and resistance genes during in situ uranium bioremediation, The ISME J., 7 (2013), 370-383.
35. Ghosh W, Dam B, Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea, FEMS Microbiol. Rev., 33 (2009), 999-1043.
36. Golyshina O. V, Ferrer M, Tindall B. J, Yakimov M. M, Nimtz M, Luensdorf H, Timmis N. K, Golyshin P. N, Wray V, Tindall B. J, *Acidiplasma aeolicum* gen. nov., sp. nov., a euryarchaeon of the family *Ferroplasmaceae* isolated from a hydrothermal pool, and transfer *of Ferroplasma cupricumulans* to *Acidiplasma cupricumulans* comb. nov., Int. J. Syst. Evol. Micro., 59 (2009), 2815-2823.
37. Gonzalez-Contreras P, Weijma J, Buisman C. J. N, Kinetics of ferrous iron oxidation by batch and continuous cultures of thermoacidophilic Archaea at extremely low pH of 1.1–1.3, Appl. Microbiol. Biotechnol., 93 (3) (2012), 1-9.
38. Gonzalez-Toril E, Llobet-Brossa E, Casamayor E. O, Amann R,Amils R, Microbial Ecology of an Extreme Acidic Environment, the Tinto River, Appl. Environ. Microbiol., 69 (8) (2003), 4853-4865.
39. Gray N.F, Environmental impact and remediation of acid mine drainage: a management problem, Environ. Geol., 30 (1997), 62-71.
40. Hallberg K. B, Johnson D. B, Biodiversity of acidophilic prokaryotes, Adv. Appl. Microbiol., 49 (2001), 37-84.
41. Hallberg K. B., Johnson D. B, Gonzalez-Toril E, *Acidithiobacillus ferrivorans*, sp. nov.; facultatively anaerobic, psychrotolerant iron-, and sulfur-oxidizing acidophiles isolated from metal mine-impacted environments, Extremophiles, 14 (2010), 9-19.

42. Hallberg K. B., Johnson D. B, Hedrich S*, Acidiferrobacter thiooxydans*, gen. nov. sp. nov.; an acidophilic,thermo-tolerant, facultatively anaerobic iron- and sulfur-oxidizer of the family *Ectothiorhodospiraceae*, Extremophiles, 15 (2011), 271-279.
43. Hao C, Wang L, Gao Y, Zhang L, Dong H, Microbial diversity in acid mine drainage of Xiang Mountain sulfide mine, Anhui Province, China, Extremophiles, 14 (2010), 465–474.
44. Hedrich S, Schlömann M, Johnson D. B, The iron-oxidizing proteobacteria, Microbiol. J., 157 (2011), 1551–1564.
45. Hedrich S, Johnson D. B, *Acidithiobacillus ferridurans* sp. nov., an acidophilic iron-, sulfur- and hydrogen-metabolizing chemolithotrophic gammaproteobacterium, Int. J. Syst. Evol. Microbiol., 63 (2013), 4018–4025.
46. Huang L.N, Zhou W.H, Hallberg K.B, Wan C.Y, Li J, Shu W.S, Spatial and temporal analysis of the microbial community in the tailings of a Pb-Zn mine generating acidic drainage, Appl. Environ. Microbiol., 77 (15) (2011), 5540-5544.
47. Huber H, Stetter K. O, Thiobacillus prosperus sp. Nov., represents a new group of halotolerant metal-mobilizing bacteria isolated from a marine geotermal field, Arch. Microbiol., 151 (1989), 479-485.
48. Jarrell K. F, Dickinson T, Walters A. D, Bochiwal C, Chong J. P. J. Borgia J. M, Major players on the microbial stage: why archaea are important, Microbiology 157 (2011), 919–936.
49. Johnson B. D, Kanao T, Hedrich S, Redox transformations of iron at extremely low pH: fundamental and applied aspects, Frontiers Microbiol., 3 (2012), 1-13.
50. Johnson D B, Geomicrobiology of extremely acidic subsurface environments, FEMS Microbiol. Ecol., 81 (2012), 2–12.
51. Johnson D. B, Biodiversity and ecology of acidophilic microorganisms, FEMS Microbiol. Ecol., 27 (1998), 307-317.
52. Johnson D. B, Biohydrometallurgy and the environment: Intimate and important interplay, Hydrothermallurgy, 83 (2006), 153-166.
53. Johnson D. B, Hallberg K. B, Carbon, iron and sulfur metabolism in acidophilic micro-organisms, Adv. Microb. Physiol., 54 (2009), 201-255.
54. Johnson D. B, Okibe N, Wakeman K, Yajie L, Effect of temperature on the bioleaching of chalcopyrite concentrates containing different concentrations of silver, Hydrometallurgy, 94 (2008), 42-47.
55. Kamimura K, Kunomura K, Wakai S, Murakami K, Sugio T, Some properties of a novel obligately autotrophic iron-oxidizing bacterium isolated from seawater, Environ. Microbiol., 59 (2001), 373-381.
56. Kappler A, Newman D. K, Formation of Fe(III)-minerals by Fe(II)-oxidizing photoautotrophic bacteria, Geochimica et Cosmochimica Acta, 68 (6) (2004), 1217–1226.
57. Kelly D. P, Wood A. P, The chemolithotrophic prokaryotes, The prokaryotes, 2 (2006), 441-456.
58. Kelly P. W, Kelly D. P, Proposal for a new class within the phylum *Proteobacteria*, *Acidithiobacillia* classis nov., with the type order *Acidithiobacillales*, and emended description of the class Gammaproteobacteria, Int. J. Syst. Evol. Micr., 63 (2013), 2901–290.

59. Kim J, Koo S.Y, Kim J.Y, Lee E.H, Lee S.D, Ko K.S, Ko D.C, Cho K. S, Influence of acid mine drainage on microbial communities in stream and groundwater samples at Guryong Mine, South Korea, Environ. Geol., 58 (2009), 1567–1574.
60. Kimm B. H, Hong B, Gadd G. M, Bacterial physiology and metabolism, Cambridge: Cambridge University Press, 2008.
61. Kock D, Schippers A, Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage. Hydrometallurgy, 83 (2006), 167–175.
62. Kock D, Schippers A, Quantitative microbial community analysis of three different sulfidic mine tailing dumps generating acid mine drainage, Appl. Environ. Microbiol. 74 (16) (2008), 5211-5219.
63. Koleini S. M. J, Jafarian M, Abdollahy M, Aghazadeh V, Galvanic leaching of chalcopyrite in atmospheric pressure and sulfate media: kinetic and surface studies, Ind. Eng. Chem. Res., 49 (2010), 5997–6002.
64. Konishi Y, Yoshida S, Asai S, Bioleaching of pyrite by acidophilic thermophile *Acidianus brierleyi*, Biotechnol. Bioeng., 48 (1995), 592-600.
65. Konishi Y, Masahiko t, Asai S, Suzuki T, Copper recovery from chalcopyrite concentrate by acidophilic thermophile *Acidianus brierleyi* in batch and continuous-flow stirred tank reactors, Hydrometallurgy, 59 (2001), 271-282.
66. Korehi H, Blöthe M, Sitnikova M A, Dold B, Schippers A, Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile, Environ. Sci. Technol. 47 (2013), 2189-2196.
67. Korehi H, Schippers A, Bioleaching of a marine hydrothermal sulfide ore with mesophiles, moderate thermophiles and thermophiles, Bioleaching of a marine hydrothermal sulfide ore with mesophiles, moderate- thermophiles and thermophiles. Adv. Mat. Res., 825 (2013), 229-232.
68. Larsson L, Olsson G, Holst O, Karlsson H T, Pyrite oxidation by thermophilic archaebacteria, Appl. Environ. Microbiol., 56(3) (1990), 697-701.
69. Gahan C. S, Sundkvist J. E, Dopson M, Sandström A, Effect of chloride on ferrous iron oxidation by a *Leptospirillum ferriphilum*-dominated chemostat culture. Biotechnol. Bioeng, 106 (3) (2010), 422-431.
70. Liu, C.-Q., Plumb, J. & Hendry, P. Rapid specific detection and quantification of bacteria and archaea involved in mineral sulfide bioleaching using real-time PCR. Biotechnol. Bioeng., 94 (2006), 330-336.
71. Mendez M. O, Neilson J. W, Maier R. M, Characterization of a bacterial community in an abandoned semiarid lead-zinc mine tailing site, Appl. Environ. Microbiol., 74 (12) (2008), 3899-3907.
72. Mousavi S. M, Yaghmaei S, Vossoughi M, Jafari A, Hoseini S.A, Comparison of bioleaching ability of two native mesophilic and thermophilic bacteria on copper recovery from chalcopyrite concentrate in an airlift bioreactor, Hydrometallurgy, 80 (2005), 139-144.

73. Niederberger T. D, Lawrence J. R, Mielke R. E, Andersen D. T, Perreault N. N, Nadeau J. L, Greer C. W, Whyte L. G, Novel sulfur-oxidizing streamers thriving in perennial cold saline springs of the Canadian high Arctic, Environ. Microbiol., 11 (3) (2009), 616-629.
74. Nordstrom, D.K., Alpers, C.N., Ptacek, C.J., Blowes, D.W., Environ. Sci. Technol., 34 (2000), 254-258.
75. Pernthaler, A., Pernthaler, J, Amann, R, Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. 68 (2002), 3094-3101.
76. Ram R. J, VerBerkmoes N. C, Thelen M. P, Tyson G. W, Baker B. J, Blake R. C, Shah M, Hettich R. L, Banfield J. F, Community proteomics of a natural microbial biofilm, science, 308 (2005), 1915-1919.
77. Rawlings D, Johnson D. B, The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia, Microbiology, 153 (2007), 315–324.
78. Rawlings D. E, Johnson D. B, The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia, Microbiology, 153 (2007), 315–324.
79. Remonsellez F, Moreno-Paz M, Acosta M, Galleguillos F, Parro V, Demergasso C, Dynamic of active microorganisms inhabiting a bioleaching industrial heap of low-grade copper sulfide ore monitored by real-time PCR and oligonucleotide prokaryotic acidophile microarray, Microbial Biotechnology, 2 (6) (2009), 613–624.
80. Robertson L. A, Kuenen J. G, The genus *Thiobacillus*, The Prokaryotes, 5 (2006), 812-827.
81. Rodriguez Y, Ballester A, Blázquez M. L, González F, Muñoz J. A, New information on the chalcopyrite bioleaching mechanism at low and high temperature, Hydrometallurgy, 71 (2003), 47-56.
82. Rogosa M, *Peptococcaceae*, a new family to include the Gram-Positive, anaerobic cocci of the genera *Peptococcus*, *Peptostreptococcus*, and *Ruminococcus*, 21 (3) (1971), 234-237.
83. Rohwerder T, Sand W, Oxidation of inorganic sulfur compounds in acidophilic prokaryotes, Eng. Life Sci., 7 (4) (2007), 301-309.
84. Sandström A, Schukarev A, Paul J, XPS characterisation of chalcopyrite chemically and bioleached at high and low redox potential, 18 (5) (2005), 505-515.
85. Schippers A, Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification. In: Donati, E, Sand W (eds), Microbial Processing of Metal Sulfides, (2007) Springer 3-33.
86. Schippers A, Bosecker K, Bioleaching: analysis of microbial communities dissolving metal sulfides. In: Barredo, J-L (Ed), Methods in biotechnology, Vol. 18: Microbial Processes and Products. J.-L. Barredo (ed.). Humana Press Inc., Totowa, NJ, USA, (2005) 405-412..
87. Schippers A, Breuker A, Blazejak A, Bosecker K, Kock D, Wright T.L, The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria, Hydrometallurgy, 104 (2010), 342–350.
88. Schippers A, Hedrich S, Vasters J, Drobe M, Sand W, Willscher S, Biomining: metal recovery from ores with microorganisms, Adv. Biochem. Eng. Biotechnol., (2014), 1-47.

89. Schippers A, Sand W, Bacterial leaching of metal sulfides poceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur, Appl. Environ. Microbiol., 65 (1) (1999), 319-321.
90. Schippers, A, Kock D, Schwartz M, Böttcher M. E, Vogel H, Hagger M, Geomicrobiological and geochemical investigation of a pyrrhotite-containing mine waste talilings dam near Selebi-Phikwe in Botswana. J. Geochem. Explor., 92 (2007), 151-158.
91. Scott K.M, Sievert S.M, et al., The genome of Deep-Sea vent chemolithoautotroph Thiomicrospira crunogena XCL-2,PLOS Biology, 4 (12) (2006), 1-47.
92. Segerer A, Neuner A, Kristjansson J. K, Stetter O.K, *Acidianus infernus* gen. nov., sp. nov. and *Acidianus brierleyi* comb. nov.: facultatively aerobic, Extremely acidophilic thermophilic sulfur-metabolizing Archaebacteria, Int. J. Syst. Bacteriol., 36 (4) (1986), 559-564.
93. Sorokin D. Y, Muyzer G, Tourova T. P, Braker G, *Thiohalomonas denitrificans* gen. nov., sp. nov. and *Thiohalomonas nitratireducens* sp. nov., novel obligately chemolithoautotrophic, moderately halophilic, thiodenitrifying Gammaproteobacteria from hypersaline habitats, Int. J. Syst. Evol. Micr., 57 (2007), 1582-1589.
94. Tan G. L, Shu W.S, Hallberg K. B, Li F, Lan C. Y, Huang L.N, Cultivation- dependent and cultivation-independent characterization of the microbial community in acid mine drainage associated with acidic Pb/Zn mine tailings at Lechang, Guangdong, China, FEMS, 59 (2007), 118-126.
95. Tan G. L, Shu W.S, Hallberg K. B, Li F, Lan C. Y, Zhou W. H, Huang L.N, Culturable and molecular phylogenetic diversity of microorganisms in an open-dumped, extremely acidic Pb/Zn mine tailings, Extremophiles, 12 (2008), 657-664.
96. Templeton A. S, Geomicrobiology of iron in extreme environmnets, Elements Journal, 7 (2011), 95-100.
97. Tyson G. W, Chapmann J, Hugenholtz P, Allen E. E, Ram R. J, Richardson P. M, Solovyev V. V, Rubin E. M, Rokhsar D. S, Banfield J. F, Community structure and metabolism through reconstruction of microbial genomes from the environment, Nature, 428 (2004), 37-43.
98. Tyson G. W, Lo I, Baker B. J, Allen E. E, Hugenholtz P, Banfield J F, Genome-directed isolation of the key nitrogen fixer *Leptospirillum ferrodiazotrophum* sp. nov. from an acidophilic microbial community, Appl. Environ. Microbiol., 71 (2005), 6319-6324.
99. Vera M, Schippers A, Sand W, Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation—part A, Appl. Microbiol. Biotechnol. 97 (2013), 7529–7541.
100. Vidyalakshmi R, Paranthaman R, Bhakzaraj R, Sulphur oxidizing bacteria and pulse nutrition-A review, World Journal of Agricultural Sciences, 5 (3) (2009), 270-278.
101. Waksman S. A, Joffe J. S, Microorganisms concerned in the oxidation of sulfur in the soil, J. Bacteriol., 7 (2) (1922), 239-256.
102. Watling H. R, Chalcopyrite hydrometallurgy at atmospheric pressure: 2. Review of acidic chloride process options, Hydrometallurgy, 146 (2014), 96-110.
103. Weber k. A, Achenbach L. A, Coates J. D, Microbes pumping iron: anaerobic microbial iron oxidation and reduction, Nat. Rev. Microbiol., 4 (2006), 752-764.
104. Winogradsky S, Über Schwefelbakterien. Botanische Zeitung 45 (1887), 489-600.

105. Wirsen C. O, Brinkhoff T, Kuever J, Muzyer G, Molyneaux S, Jannasch H. W, Comparison of a new *Thiomicrospira* strain from the mid-atlantic ridge with known hydrothermal vent isolates, Appl. Environ. Microbiol., 64 (10) (1998), 4057-4059.
106. Yoon J.H, Kang S. J, Oh T.K, *Polaribacter dokdonensis* sp. nov., isolated from seawater, Int. J. Syst. Evol. Micr., 56 (2006), 1251–1255.

2.4. Manuscript overview

Chapter 3

Hananeh Korehi, Marco Blöthe, Maria A. Sitnikova, Bernhard Dold and Axel Schippers. 2013. Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile. *Environ. Sci. Technol*. 47, 2189-2196.

Author contributions: A.S. and B.D. designed the study and performed sample collection. H.K. carried out the microbiological investigations. M.B. contributed to phylogenetic analyses. M.A.S. performed SEM-MLA analyses. H.K. and A.S. wrote the manuscript.

Chapter 4

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Chapter 5

Hananeh Korehi and Axel Schippers. 2013. Bioleaching of a marine hydrothermal sulfide ore with mesophiles, moderate thermophiles and thermophiles. Advanced Materials Research 825, 229-232.

Author contributions: A.S. designed the study. H.K. carried out the microbiological investigations. H.K. and A.S. wrote the manuscript.

Chapter 3

Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile *(Environ. Sci. Technol*. 47 (2013) 2189-2196)

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Abstract

The marine shore sulfidic mine tailings dump at the Chanaral Bay in the Atacama Desert, northern Chile, is characterized by extreme acidity, high salinity, and high heavy metals concentrations. Due to pyrite oxidation, metals (especially copper) are obilized under acidic conditions and transported toward the tailings surface and precipitate as secondary minerals (Dold, Environ. Sci. Technol. 2006, 40, 752−758.). Depth profiles of total cell counts in this almost organic-carbon free multiple extreme environment showed variable numbers with up to 108 cells g^{-1} dry weight for 50 samples at four sites. Real-time PCR quantification and bacterial 16S rRNA gene diversity analysis via clone libraries revealed a dominance of Bacteria over *Archaea* and the frequent occurrence of the acidophilic iron(II)- and sulfur-oxidizing and iron(III)-reducing genera *Acidithiobacillus*, *Alicyclobacillus*, and *Sulfobacillus*. Acidophilic chemolithoautotrophic iron(II)-oxidizing bacteria were also frequently found via most-probable-number (MPN) cultivation. Halotolerant iron(II)-oxidizers in enrichment cultures were active at NaCl concentrations up to 1M. Maximal microcalorimetrically determined pyrite oxidation rates coincided with maxima of the pyrite content, total cell counts, and MPN of iron(II)-oxidizers. These findings indicate that microbial pyrite oxidation and metal mobilization preferentially occur in distinct tailings layers at high salinity. Microorganisms for biomining with seawater salt concentrations obviously exist in nature.

3.1. Introduction

Extreme environments on Earth such as deep sediments and rocks, hot springs, acid mine drainages, or salt lakes have shown to harbor active and specialized microbial communities. The hyperarid Atacama Desert is one of the driest deserts on Earth but is still a habitat for microorganisms (6-7). Microorganisms play a significant role in

metallogenetic processes in dumps of mine waste rock and tailings from sulfide ore processing plants. Acidophilic iron(II)- and sulfur-oxidizing bacteria are responsible for the release of sulfuric acid and dissolved metals such as iron, copper, nickel, zinc, and arsenic, known as acid mine drainage from such dumps by catalyzing the oxidation of metal sulfides, mainly pyrite (or pyrrho-tite) (8-19). Over a period of several years, an oxidized zone with depleted metal sulfide content, low pH, and enrichment of secondary minerals is developing above a not oxidized zone with unaltered material in the waste dump. Anaerobic iron(III)- and sulfate-reducing bacteria have been detected as well in several mine dumps enabling a complete biogeochemical iron-and sulfur-cycle, which is important for long-term (bio)remediation (8-17). The microbial metal sulfide oxidation processes are also used for biomining, an industrial recovery of copper, cobalt, nickel, zinc, gold, and uranium via dump or heap bioleaching (20,21). High concentrations of chloride ions inhibit the growth of acidophilic microorganisms used in biomining, a problem particularly relevant to Australian and Chilean biomining operations (22). Few species of iron- and sulfur-oxidizing bacteria grow in saline, strongly acidic environments; however, copper ore bioleaching with halotolerant microorganisms has been demonstrated in the laboratory (23). In the best case seawater should be used for biomining operations to save freshwater resources, but microbial metal sulfide oxidation at seawater salt concentrations and low pH has not been shown yet in the environment, such as sulfidic mine tailings. Microorganisms were found so far only in sulfidic mine waste rock and tailings at humid and semiarid conditions but not under the multiple extreme conditions of the Atacama Desert to the best of our knowledge (8). The extreme acidic, metal-rich, and high-saline, sulfidic mine tailings dump at the Chañaral Bay in the Atacama Desert originated from copper ore processing (1938-1975) and was classified by the United Nations Environmental

Programme (UNEP) in 1983 as one of the most serious contamination sources in the Pacific area. Tailings with an average pyrite content of 0.8 wt % were deposited into the bay, in total over 220 Mt. This resulted in a 10-15 m thick tailings dump covering about 4 km^2 and in a more than 1km seaward displacement of the shoreline. The tailings material has been exposed to oxidation since 1975 which resulted in a 70-188 cm thick low-pH (2-4) oxidation zone. The redox potential (Eh) showed values of up to 600 mV, and NaCl concentrations of up to more than 1M were measured in the pore water of the oxidation zone. Elevated pore water concentrations of up to more than 6000 mg/L sulfate, 2265 mg/L copper, 20 mg/L zinc, and 18 mg/L nickel were found. Evaporation-induced upward transport of metals led to metal enrichment at the tailings surface and secondary chlorides and/ or sulfates precipitated (e.g., up to 2.4% Cu). The mainly water soluble, secondary minerals have been transported by the wind, also toward the village of Chañaral (Supplementary Figure S1; ref 1). In this study an existence of microorganisms, their identification, and quantification as well as their impact on pyrite oxidation at high salinity and low pH relevant for copper mobilization have been explored.

3.2. Methods

3.2.1. Tailings sampling

In November 2008, 50 samples were taken down to a maximal depth of 105 cm from outcrop profiles at four sites of the tailings dump with sterile spatulas or spoons and filled in sterile 100 mL containers. Site CH1 was located at the southern part, sites CH11 and CH12 at the central part, and site CH14 at the northern part of the tailings dump. Site CH12 was located close to the sea shore (beach). The paste pH was measured in the field with an electrode after shaking of 5 g tailings material in 12.5 mL of 1 M KCl for 5 min. The samples were transported to the BGR geomicrobiology

laboratory within a couple of days for further analyses. Immediately after arrival the samples were split in subsamples which were either instantly used for geochemical and microcalorimetric measurements and inoculation of media for cultivation, or fixed with formaldehyde for total cell counts and CARD-FISH, or frozen at -20 °C for later analyzes with DNA-based techniques. Geochemistry and Mineralogy. Humidity was determined as weight difference after drying of 5 g tailings at 105 °C. The mineralogy was quantitatively analyzed by an Environmental Scanning Electron Microscope (ESEM, type FEI Quanta 600 FEG) coupled with an energy dispersive X-ray (EDX) detector (Apollo XL from Ametek Inc.) and in combination with the MLA software package (Mineral Liberation Analyzer, FEI). The investigated polished sections were measured by the XBSE method (24) with HV 25 Kv and 5.7 µm beam spot size. Total element analysis was done as previously described (XRF and LECO; ref 16).

3.2.2. Pyrite oxidation rates

The potential pyrite oxidation rate at atmospheric oxygen partial pressure was determined by microcalorimetry as described (12, 14-16, 25, 26) because the reaction rate correlates with the heat output. A complete oxidation of pyrite to iron(III) and sulfate produces a reaction energy of -1546 kJ/mol:

$$FeS_2 + 3.75O_2 + 0.5H_2O \rightarrow Fe^{+3} + 2SO_4^{-2} + H^+, \quad \Delta f\, H0 = -1/1.546\ (\text{mmol/kJ}) \qquad (1)$$

The pyrite oxidation rate r (µg/kg/s) was calculated using the transformed reaction energy value of -1.546 kJ/mmol, the molecular mass of pyrite of 0.12 kg/mol, the measured heat output a (µW), and the sample weight w (g) by the following equation:

r (µg/kg/s) = 1/-1.546 (mmol/kJ) × 0.12 (kg/mol) × a (µW) × 1/w (1/g) (2)

After measuring the total rate (chemical plus biological), the bacteria were inactivated by heating to 60°C for ca. 12 h, and the chemical rate was measured afterward. The inactivation of mesophilic pyrite-oxidizers was already described and checked via cultivation (25). The biological rate was calculated as the differenece of both measurements.

3.2.3. Cultivation

Microorganisms were detected and quantified by cultivation and molecular techniques as described previously (16). The most-probable-number (MPN) cultivation technique was used to enumerate acidophilic chemolithoautotrophic iron(II)- and sulfur-oxidizing bacteria (26). Aerobic acidophilic heterotrophs were enumerated on agar plates (12). Acidophilic halotolerant iron(II)-oxidizers were enriched and cultivated in 100 mL Erlenmeyer flasks in 50 mL medium supplemented with 1g L^{-1} Fe^{2+} as $FeSO_4$ and sodium chloride concentrations of 0.5 and 1 M at 30 °C on a rotary shaker with a rotation speed of 120 rpm (26). In the porewater from the oxidized tailings NaCl concentrations of up to more than 1 M were measured (1), thus our medium mimicked field salinity. Besides, the enrichment medium consisted of the following: 0.15 g/L $(NH_4)_2$ SO_4 , 0.05 g/L KCl, 0.5 g/L $MgSO_4$ ×7 H_2O, 0.05 g/L K_2HPO_4, and 0.01 g/L $Ca(NO_3)_2$ × $4H_2O$, adjusted to pH 3.5 with H_2SO_4 or NaOH. Water evaporation was compensated at regular intervals by addition of acidic deionized water during the experiments, and samples were taken at different time intervals. Growth was checked by phase contrast microscopy, the pH was measured, and the iron(II)-oxidation activity was monitored by regular Fe^{2+} and total Fe measurement by the o-phenanthroline colorimetric method.

3.2.4. Total cell counts and CARD-FISH

Total cell numbers were determined in formaldehyde-fixed samples by staining with SYBR Green II following two different protocols. On the one hand cells were counted directly in the samples; on the other hand cells were detached from tailings particles before counting using a different protocol (27). The highest number of the two counts is reported here since an overestimation of counts is unlikely. CARD-FISH analysis was carried out as previously done with formaldehyde fixed samples, and filters were hybridized for *Archaea* and *Bacteria* using probes ARCH915 or EUB338 I-III as a mixture (3, 16, 28-30). As a negative hybridization control the probe NON338 was applied and cell signals were not detected. The formamide concentrations were 55% for all probes. DAPI was used for counterstaining.

3.2.5. Real-Time PCR

DNA was extracted from 0.5 g of a frozen tailings sample following a modified Fast DNA Spin Kit for Soil (Bio 101) protocol (31). This protocol has shown to exhibit the highest DNA copy numbers from tailings samples among several others tested. DNA extracts from blank tubes (no sample added) were used as negative control in the extraction procedure. Extracted DNA was amplified by qPCR using the device ABI Prism 7000 (Applied Biosystems) and master mixes from the companies Applied Biosystems, Eurogentec, or Invitrogen. Each DNA extract was measured in triplicate. The copy numbers of the 16S rRNA gene were quantified for *Archaea* (32) and *Bacteria* (33) based on the TaqMan chemistry. The specific bacterial 16S rRNA genes of *Acidithiobacillus* spp., *Leptospirillum* spp. (35), and *Sulfobacillus* spp.(35), and the functional *dsr*A gene of sulfate-reducers (36) were also quantified using qPCR with SYBR Green I chemistry. After each qPCR, melting curves were measured.

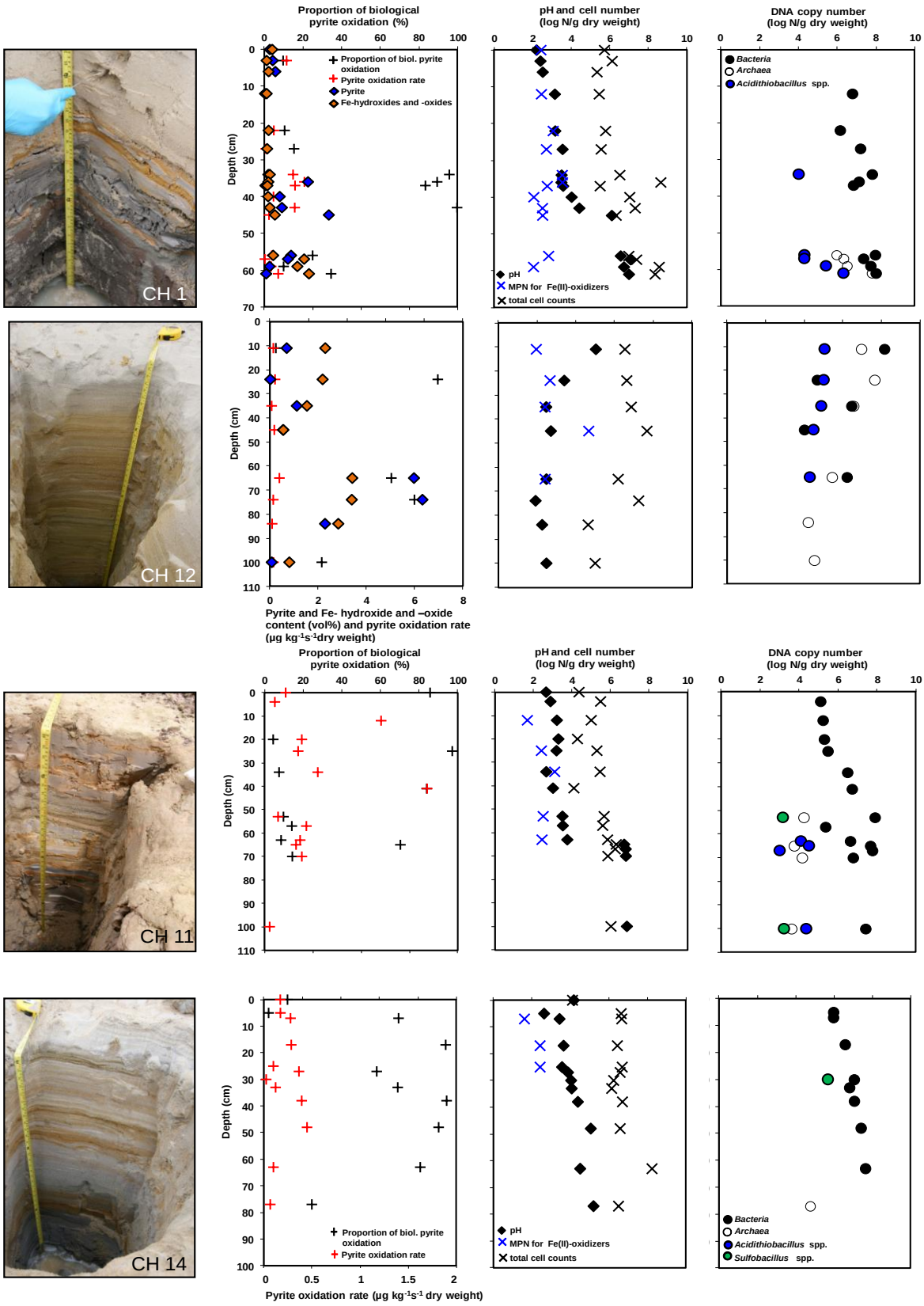

Figure 1. Pictures of outcrop profiles and depth dependent quantitative mineralogy, pyrite oxidation activity, and quantitative microbial community analysis for the sampling sites of the high-saline, sulfidic mine tailings dump at Chañaral, Atacama Desert, northern Chile. From top to bottom data for the sites are shown in the order CH1, CH12, CH11, and CH14.

The primer specificity for the specific qPCR assays was confirmed by sequence alignment in databases (Blast, Ribosomal Database Project). The detection limits for qPCR analyses were 10^3 16S rRNA gene copies g^{-1} dry weight (dw) for the assays specific for *Bacteria* and *Acidithiobacillus* spp., 10^2 copies g^{-1} dw for the assays specific for *Leptospirillum* spp. and *dsr*A, and 10^1 copies g^{-1} dw for the assays specific for *Archaea* and *Sulfobacillus* spp..

3.2.6. Microbial diversity

The amplification of 16S rRNA genes from Bacteria was performed by PCR with the universal bacterial primers GM3F (5'-AGAGTTTGATCMTGGC-'3, position 8 to 24) and GM4R (5'-TACCTTGTTACGACTT'3, position 1492 to 1507). 37 PCR mix was prepared from Thermo Scientific 2xMasterMix (final concentration: 75 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 0.2 mM each of dNTP, 0.5 µM each of primer, 0.652 U ThermoPrime Taq DNA Polymerase, 100 ng/µL BSA) and a 2 µL template of extracted DNA (see above) in a total reaction of volume 50 µL. Negative controls without template were used as a contamination check. Reaction mixtures were held at 95 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 90 s, with a final extension step of 5 min at 72 °C. Products of PCR reactions were cloned and sequenced by the company Microsynth (Switzerland). Overlapping sequencing from both sides of the 16S rRNA genes was performed. Contigs were constructed with the software Geneious Pro 5.4 and checked for chimera with UCHIME (37). In total 800 sequences with more than 1300 bp were obtained (Supplementary Table S4). Obtained sequences were aligned with the SILVA Incremental Aligner (SINA 38) and the SILVA_108NR database and curated by hand with the ARB software package (v.5.4). This alignment was used in the Mothur v 1.29 program to build operational

taxonomic units (OTU, 97% similarity) and calculate coverage and diversity indices. One sequence from each OTU harboring at least 5 sequences was picked as a representative and imported to the SILVA_108NR template tree 39 using the ARB program suite (40). An additional 10 sequences for each OUT representatitive were selected based on the phylogenetic affiliation (min identity 5%, in total 300) in the SILVA_108NR. Selected reference sequences together with the OUT representatives were used for tree construction using maximum likelihood algorithm (RAxML) with GTRGAMMA as rate distribution model and the general bacteria filter provided in ARB. The rRNA gene sequences obtained in this study were submitted to the European Nucleotide Archive with the accession numbers HF558531-HF558644.

3.3. Results and discussion

The extreme acidic, metal-rich, and high-saline, sulfidic mine tailings dump at the Chañaral Bay in the Atacama Desert with copper enrichment at the surface is the most extreme mine tailings studied so far. The copper enrichment zone is particularly interesting for mining of this secondary copper resource. In November 2008, 50 samples were taken from different depths of the oxidation zone above the seawater level at four sites of the Chañaral mine tailings. The geochemistry and mineralogy of these sites was previously studied in particular (1). Here, in addition the mineralogy has quantitatively been analyzed by scanning electron microscopy – mineral liberation analysis (SEM-MLA, Figure 1 and Supplementary Table S1) together with total element analysis (XRF and LECO, Supplementary Table S2). Organic carbon was below the detection limit of 0.01% at all sites (besides at about 60 cm depth of site CH1 where a buried alluvion (soil material) and no tailings material was sampled). Thus the substrate for heterotrophic microorganisms usually found in extreme environments is absent in the tailings. According to the quantitative mineralogical

analysis, pyrite is the main substrate for chemolithoautotrophic microorganisms. In Figure 1, depth profiles of pyrite and its oxidation product iron hydroxides and oxides are shown together with pyrite oxidation rates as well as the proportion of the biological versus the chemical pyrite oxidation rate determined by microcalorimetric measurements. A coincidence of the maxima of the pyrite content and the pyrite oxidation rate was found. The pyrite maxima at ~35-45 cm depth for CH1 and ~60-80 cm depth for CH12 coincided with a high proportion of biological pyrite oxidation indicating current oxidation activity there. Microorganisms were detected and quantified by cultivation as well as molecular techniques (16). Depth profiles of cell numbers together with pH-values and pictures of the four sampled depth profiles are shown in Figure 1. The pictures of all sites show alternating layers of gray and brown colors reflecting the deposition history of the tailings as well as a variable precipitation of iron hydroxides and oxides as secondary minerals due to mainly pyrite oxidation. Living acidophilic chemolithoautotrophic iron(II)-oxidizing microorganisms able to oxidize pyrite were detected via cultivation in liquid media in variable most probable numbers (MPN) at all sites in half of the samples (25 of 50 samples). The highest MPN numbers were detected in tailings layers with a pH 2-4. Acidophilic chemolithoautotrophic sulfur-oxidizing microorganisms and acidophilic heterotrophs were scarcely detected via cultivation (6 of 50 and 5 of 50 samples, respectively, data not shown). Thus the MPN cultivation approach likely missed most of these organisms. The heterotrophs presumably exist from little organic carbon released from the chemolithotrophs as previously shown for sulfidic mine waste (8,12). Total cell counts determined by counting under a fluorescence microscope after DNA-staining with SYBR Green occurred in orders of magnitude higher numbers than the iron(II)-oxidizers. This means that the MPN cultivation approach covered only a minor part of

the microbial community. However, the maxima of pyrite content and pyrite oxidation activity coincided with the maxima of total cell counts and MPN numbers of acidophilic iron(II)-oxidizers. This proposes that pyrite is oxidized by the microorganisms in these tailings layers.

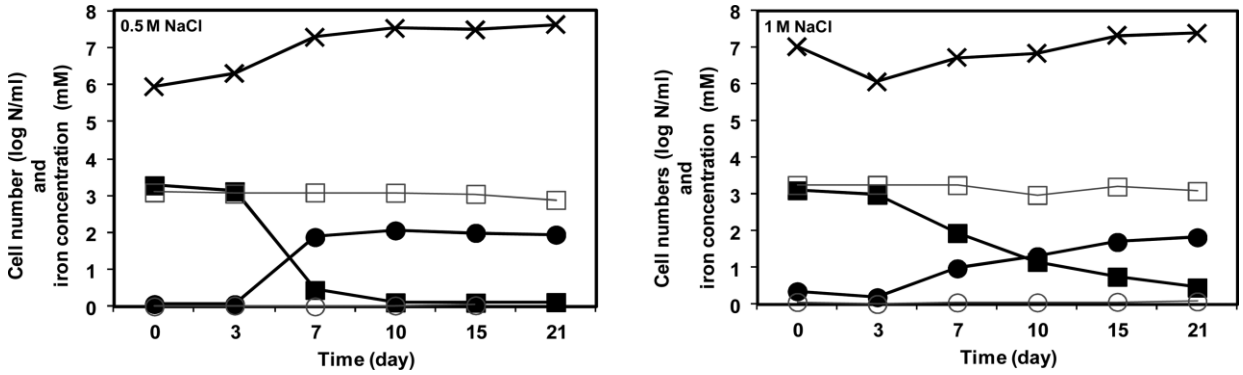

Figure 2. Growth of halotolerant, acidophilic, chemolithoautotrophic Fe(II)-oxidizing bacteria at NaCl concentrations of 0.5 and 1 M. Cell numbers (X), Fe(II) concentration (■,□) and Fe(III) concentration (●,○). Full and open symbols represent enrichment cultures and chemical controls, respectively.

In order to proof that iron(II) is indeed microbiologically oxidized at high salinity, enrichment cultures at salt concentrations of 0.5 and 1 M NaCl were set up. The mixed enrichment cultures have shown to be stable in several transfers to fresh medium. A slow growth was observed in a period of three weeks and iron(II)-oxidation was measurable only in the presence of bacteria as shown for one transfer of the most active culture (Figure 2). This culture was initially inoculated with tailings from site CH1 at about 35 cm depth, the tailings layer with the highest pyrite oxidation rates and total cell counts (Figure 1). Most of the known acidophilic iron(II)-oxidizing microorganisms are inhibited by chloride ions (22). To the best of our knowledge this is the first report of a halotolerant, acidophilic iron(II)-oxidizing culture able to oxidize iron(II) at the high salt concentration of 1 M NaCl, higher than in a previous study with the halotolerant

iron(II)-oxidizer *Thiobacillus prosperus* which oxidized iron(II) in the presence of up to 5% w/v NaCl concentration (23,41). Phylogenetic analysis based on 16S rRNA gene isolation and sequencing revealed an affiliation of the halotolerant bacteria in our stable, most active enrichment culture to *Sulfobacillus* spp. and *Acidiphilium* spp. (Supplementary Tables S3 and S4).

Also, living bacteria were detected by catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) in samples from the mine tailings selected based on the brightness of the signals (Figure S2). CARD-FISH signals were not quantified due to the low number of bright cell signals in many samples; here the data just confirm that living Bacteria exist in the multiple extreme mine tailings. A molecular quantification of microorganisms using quantitative real-time PCR (qPCR) assays allowed a comparative quantitative microbial community analysis in the Chañaral tailings dump (Figure 1). At all sampling sites, the 16S rRNA gene copy numbers (10^4-10^8 copies g^{-1}dry weight) of the domain *Bacteria* were higher than those of the domain *Archaea* which were detected in fewer samples (36 of 50 and 15 of 50 samples, respectively). In other mine tailings most detected genera belong to the domain *Bacteria* (8). The *Archaea* are mainly thermophiles (8), but high temperatures have not been observed in the Chañaral tailings dump. Thus, *Archaea* are obviously of minor importance in this multiple extreme environment. To further explore the quantitative bacterial community composition relevant for mine tailings, additionally, iron(II)and sulfur-oxidizing bacteria and anaerobic iron(III)- and sulfate-reducing bacteria were analyzed via specific qPCR assays (16).

The acidophilic iron(II)- and sulfur-oxidizing and iron(III)-reducing genera *Acidithiobacillus* (gram-negative) and *Sulfobacillus* (gram-positive) were detected in three and two sampling sites, respectively, in specific tailings layers with up to 10^5 16S

rRNA gene copies g^{-1} dry weight (Figure 1) The acidophilic Fe(II)-oxidizing genus *Leptospirillum* was below detection limit of 10^2 16S rRNA gene copies g^{-1} dry weight. A dominance of *Acidithiobacillus* over *Sulfobacillus* and *Leptospirillum* was also previously found for three other mine tailings sites (16). Sulfate-reducers were mainly detectable in the buried alluvion of site CH1 where neutral pH and little organic carbon prevailed (4 of 50 samples, data not shown). Thus, sulfate-reducers do not play a relevant role in these extreme tailings as reported for other tailings (16-17).

To further explore the microbial diversity in the tailings dump, the 16S rRNA genes of Bacteria from the samples from the sites CH1 and CH12 were amplified by PCR. Products were obtained for seven samples, cloned, and subsequently sequenced. The results are shown in Figure 3 and the Supplementary Tables S3, S4, and S5. In total 114 operational taxonomic units (OTUs, potentially representing bacterial species) were detected. Site 1 clone libraries from the depth interval 34-57 cm showed high values for recovery between 88 and 98% which correlated with the low number of observed OTUs. Relative abundant sequences were closely related to strains of the acidophilic iron(II)- and sulfur-oxidizing and

iron(III)-reducing *Alicyclobacillus* spp. (*Firmicutes*, closely related to *Sulfobacillus*) and *Acidithiobacillus ferrooxidans* (*Gammaproteobacteria*) in agreement with the qPCR data and previous tailings studies (8).

In addition, moderate acidophilic sulfur-oxidizing *Halothiobacillus* spp., *Thiohalomonas* spp., *Thiomicrospira* spp., and *Bacteriodetes* were often found in the clone library data. These particular bacterial groups have not been reported to occur in other mine tailings (8); however, moderate acidophilic sulfur-oxidizing bacteria are regularly found in sulfidic mine waste at slightly acidic or neutral pH, where due to chemical pyrite

oxidation sulfur compound intermediates are delivered as substrates for these microorganisms (9,12,15).

In the buried alluvion of site CH1 (~60 cm depth) where, with neutral pH and little organic carbon availability, less extreme conditions prevailed, the microbial diversity was much higher, and unknown representatives of the phyla *Alphaproteobacteria*, *Gmmaproteobacteria* (including *Acidithiobacillus ferrooxidans* probably introduced.

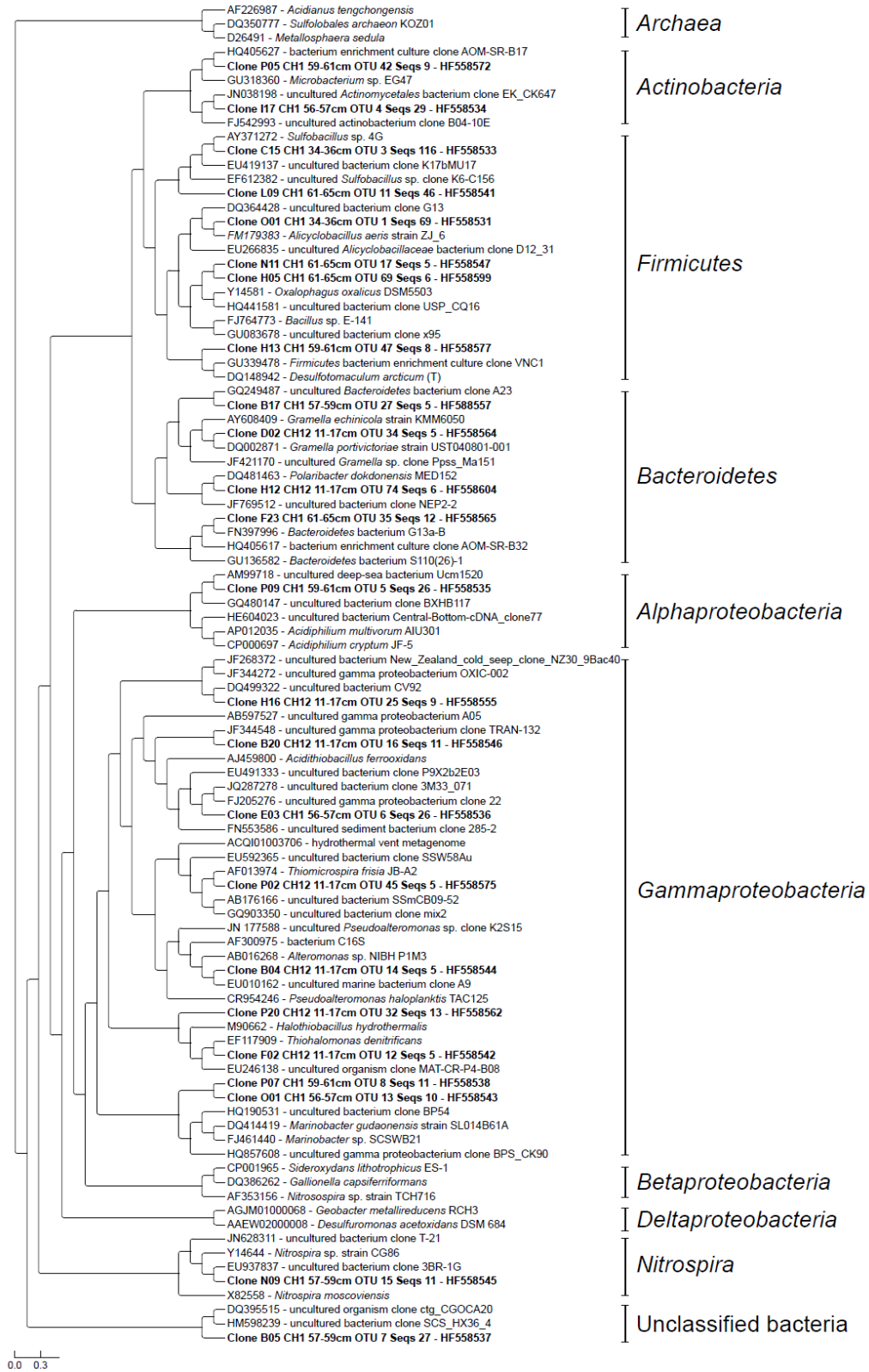

Figure 3. Maximum-likehood phylogenetic tree of abundant 16S rRNA gene sequences isolated from selected samples from the multiple extreme sulfidic mine tailings dump at Chañaral. Represented in bold is one representative sequence from each defined OUT which contained at least five assigned sequences. The scale bar indicates the number of substitutions per site.

from upper horizons), *Deltaproteobacteria* (presumably sulfate-reducers according to qPCR data), *Bacteriodetes*, *Nitrospira*, *Firmicutes*, and Actinobacteria were found. Clone libraries from these depths showed lower values for coverage between 73 and 78%. This study has shown that the multiple extreme arid, highsaline, metal-rich, and almost organic carbon-free mine tailings are populated by prokaryotes dominated by halotolerant, acidophilic, iron- and sulfur-oxidizing chemolithoautotrophic bacteria. The maximum abundance of these bacteria coincided with a high proportion of biological pyrite oxidation, maximal pyrite oxidation rates as well as a high pyrite content in distinct layers. These findings indicate that pyrite oxidation is driven by microbial activity in the extreme mine tailings. Due to microbial pyrite oxidation sulfuric acid in generated, and metals (mainly copper) are mobilized under acidic conditions which is reflected by the elevated metal and sulfate concentrations in the pore water (1). Driven by evaporation the ions are transported toward the tailings surface where metal precipitation occurs in the form of secondary chlorides and/or sulfates (1). The metal enrichment in distinct tailings horizons is potentially relevant for copper mining from mine waste. One processing option is biomining using halotolerant, acidophilic, pyrite-oxidizing chemolithoautotrophic bacteria.

Associated content
Supporting Information
Supplementary Figures S1-S3, Supplementary Tables S1-S5, and references. This material is available free of charge via the Internet at http://pubs.acs.org.

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A.S. and B.D. designed the study and performed sample collection. H.K. carried out the microbiological investigations. M.B. contributed to phylogenetic analyses. M.A.S. performed
SEM-MLA analyses. H.K. and A.S. wrote the manuscript.

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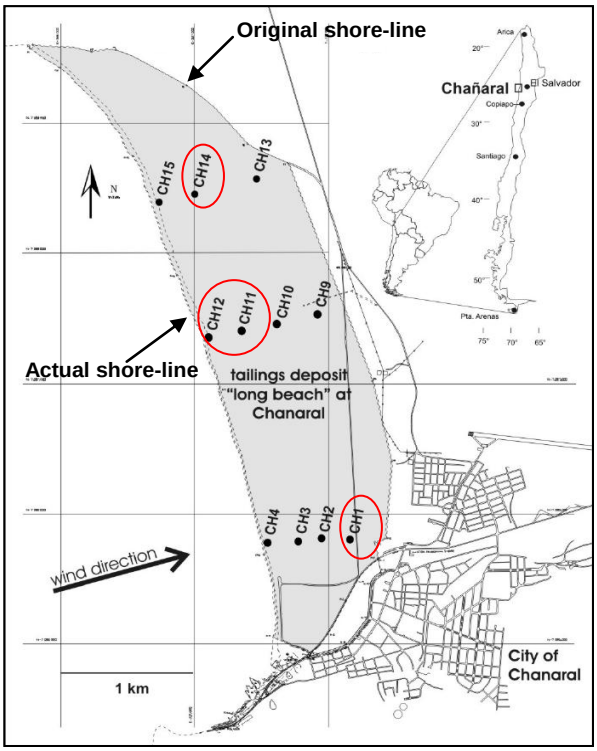
References

1. Dold, B. Element flows associated with marine shore mine tailings deposits. Environ. Sci. Technol. 2006, 40, 752-758.
2. Rothschild, L. J.; Mancinelli, R. L. Life in extreme environments. Nature 2001, 409, 1092-1101.
3. Schippers, A.; et al. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. Nature 2005, 433, 861-864.
4. Edwards, K. J.; Bond, P. L.; Gihring, T. M.; Banfield, J. F. An archaeal iron-oxidizing extreme acidophile important in acid mine drainage. Science 2000, 65, 1796-1799.
5. Chivian, D.; et al. Environmental genomics reveals a single species ecosystem deep within Earth. Science 2008, 322, 275-278.
6. Navarro-Gonzalez, R.; et al. Mars-like soils in the Atacama Desert, Chile, and the dry limit of Microbial. life. Science 2003, 302, 10181021.
7. Orlando, J., Caru, M., Pommerenke, B.; Braker, G. Diversity and activity of denitrifiers of Chilean arid soil ecosystems. Frontiers Microbiol. 20012, 3, 101.
8. Schippers, A.; et al. The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria. Hydrometallurgy 2010, 104, 342-350.
9. Schippers, A. In Sulfur Biogeochemistry - Past and Present; Special Paper, 379; Amend, J. P., Edwards, K. J., Lyons, T. W., Eds.; Geological Society of America: Boulder, Colorado, USA, 2004; pp 49-62.
10. Blowes, D. W.; Ptacek, C. J.; Jurjovec, J. In Environmental Aspects of Mine Wastes; Jambor, J. L., Blowes, D. W., Ritchie, A. I. M., Eds.; Mineralogical Association of Canada: Vancouver, BC, Canada, 2003; Vol. 31, Chapter 5, Short Course Series, pp 95-116.
11. Herbert, R. B., Jr.; Schippers, A. Iron isotope fractionation by biogeochemical processes in mine tailings. Environ. Sci. Technol. 2008, 42, 1117-1122.
12. Schippers, A.; Hallmann, R.; Wentzien, S.; Sand, W. Microbial diversity in uranium mine waste heaps. Appl. Environ. Microbiol. 1995, 61, 2930-2935.
13. Diaby, N.; et al. Microbial communities in a porphyry copper tailings impoundment and their impact on the geochemical dynamics of the mine waste. Environ. Microbiol. 2007, 9, 298-307.
14. Schippers, A.; et al. Geomicrobiological and geochemical investigation of a pyrrhotite-containing mine waste talilings damnear Selebi-Phikwe in Botswana. J. Geochem. Explor. 2007, 92, 151-158.
15. Schippers, A.; Jozsa, P. G.; Sand, W.; Kovacs, Z. M.; Jelea, M. Microbiological pyrite oxidation in a mine tailings heap and its relevance to the death of vegetation. Geomicrobiol. J. 2000, 17, 151162.
16. Kock, D.; Schippers, A. Quantitative microbial community analysis of three different sulfidic mine tailings dumps generating acid mine drainage. Appl. Environ. Microbiol. 2008, 74, 5211-5219.

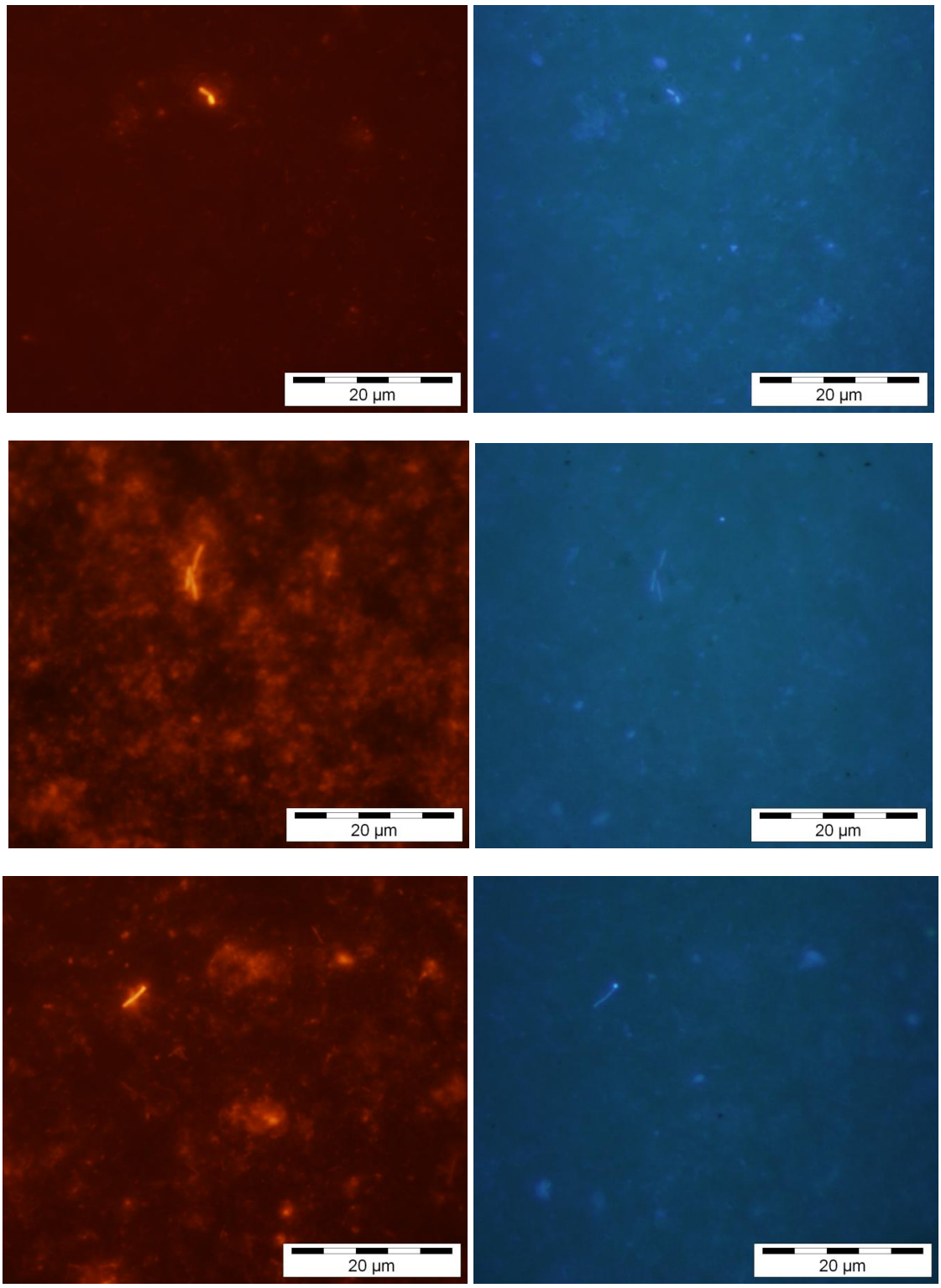
17. Praharaj, T.; Fortin, D. Seasonal variations of microbial sulfate and iron reduction in alkaline Pb-Zn mine tailings (Ontario, Canada). Appl. Geochem. 2008, 23, 3728-3740.
18. Dold, B. Sustainability in metal mining: from exploration, over processing to mine waste management. Rev. Environ. Sci. Biotechnol 2008, 7, 275-285.
19. Dold, B.; Diaby, N.; Spangenberg, J. E. Remediation of a marine shore tailings deposit and the importance of water-rock interaction on element cycling in the coastal aquifer. Environ. Sci. Technol. 2011, 45, 4876-4883.
20. Donati, E. R.; Sand, W. Microbial Processing of Metal Sulfides; Springer: Dordrecht, The Netherlands, 2007.
21. Rawlings, D. E.; Johnson, D. B. Biomining; Springer: Berlin, 2007.
22. Zammit, C. M.; Mangold, S.; Rao Jonna, V.; Mutch, L. A.; Watling, H. R.; Dopson, M.; Watkin, E. L. J. Bioleaching in brackish waters-effect of chloride ions on the acidophile population and proteomes of model species. Appl. Microbiol. Biotechnol. 2012, 93, 319-329.
23. Davis-Belmar, C. S.; Nicolle, J.; Le, C.; Norris, P. R. Ferrous iron oxidation and leaching of copper ore with halotolerant bacteria in ore columns. Hydrometallurgy 2008, 94, 144-147.
24. Redwan, M.; Rammlmair, D.; Meima, J. A. Application of mineral liberation analysis in studying micro-sedimentological structures within sulfide mine tailings and their effect on hardpan formation. Sci. Total Environ. 2012, 414, 480-493.
25. Rohwerder, T.; Florian, B. M.; Bellenberg, S.; Wentzien, S.; Sand, W. Monitoring activities of leaching microorganisms at coal mining sites. In Coal mining: research, technology and safety; Fosdyke, G. B., Ed.; Nova Science Publishers: Hauppauge, New York, 2008; pp 97118.
26. Schippers, A.; Bosecker, K. Bioleaching: Analysis of microbial communities dissolving metal sulphides. In Microbial Processes and Products, Methods in Biotechnology; Barredo, J. L., Ed.; Humana Press: Totowa, NJ, 2005; Vol. 18, pp 405-412
27. Kallmeyer, J.; Smith, D. C.; Spivack, A. J.; D Hondt, S. L. New cell extraction procedure applied to deep subsurface sediments.Limnol. Oceanogr. Methods 2008, 6, 236-245.
28. Pernthaler, A.; Pernthaler, J.; Amann, R. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. 2002, 68, 3094-3101.
29. Stahl, D. A.; Amann, R. Development and application of nucleic acid probes. In Nucleic Acid Techniques in Bacterial Systematics; Stackebrandt, E., Goodfellow, M., Eds.; John Wiley & Sons Ltd.: Chichester, UK, 1991; pp 205-248.
30. Daims, H.; Bruhl, A.; Amann, R.; Schleifer, K.-H.; Wagner, M. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: Development and evaluation of a more comprehensive probe set. System Appl. Microbiol. 1999, 22, 434-444.
31. Webster, G.; Newberry, C. J.; Fry, J.; Weightman, A. J. Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: a cautionary tale. J. Microbiol. Methods 2003, 55, 155-164.
32. Takai, K.; Horikoshi, K. Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. Appl. Environ. Microbiol. 2000, 66, 5066-5072.

33. Nadkarni, M.; Martin, F. E.; Jacques, N. A.; Hunter, N. Determination of bacterial load by real-time PCR using a broad range (universal) probe and primer set. Microbiology 2002, 148, 257-266.
34. Zammit, C. M.; Mutch, L. A.; Walting, H. R.; Watkin, E. L. J. Evaluation of quantitative real time polymerase chain reaction for enumeration of biomining microorganism in culture. Hydrometallurgy 2008, 94, 185-189.
35. Liu, C.-Q.; Plumb, J.; Hendry, P. Rapid specific detection and quantification of bacteria and archaea involved in mineral sulfide bioleaching using real-time PCR. Biotechnol. Bioeng. 2006, 94, 330336.
36. Schippers, A.; Neretin, L. N. Quantification of microbial communities in near-surface and deeply buried marine sediments on the Peru continental margin using real-time PCR. Environ. Microbiol. 2006, 8, 1251-1260.
37. Edgar, R. C.; et al. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 2011, 27, 2194-2200.
38. Pruesse, E.; Peplies, J.; Glockner, F. O. SINA: Accurate high throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 2012, 28, 1823-1829.
39. Pruesse, E.; Quast, C.; Knittel, K.; Fuchs, B. M.; Ludwig, W.; Peplies, J. SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 2007, 35, 7188-7196.
40. Ludwig, W.; et al. ARB. A software environment for sequence data. Nucleic Acids Res. 2004, 32, 1363-1371.
41. Huber, H.; Stetter, K. O. Thiobacillus prosperus sp. nov., represents a new group of halotolerant metal-mobilizing bacteria isolated from a marine geothermal field. Arch. Microbiol. 1989, 151, 479-485.

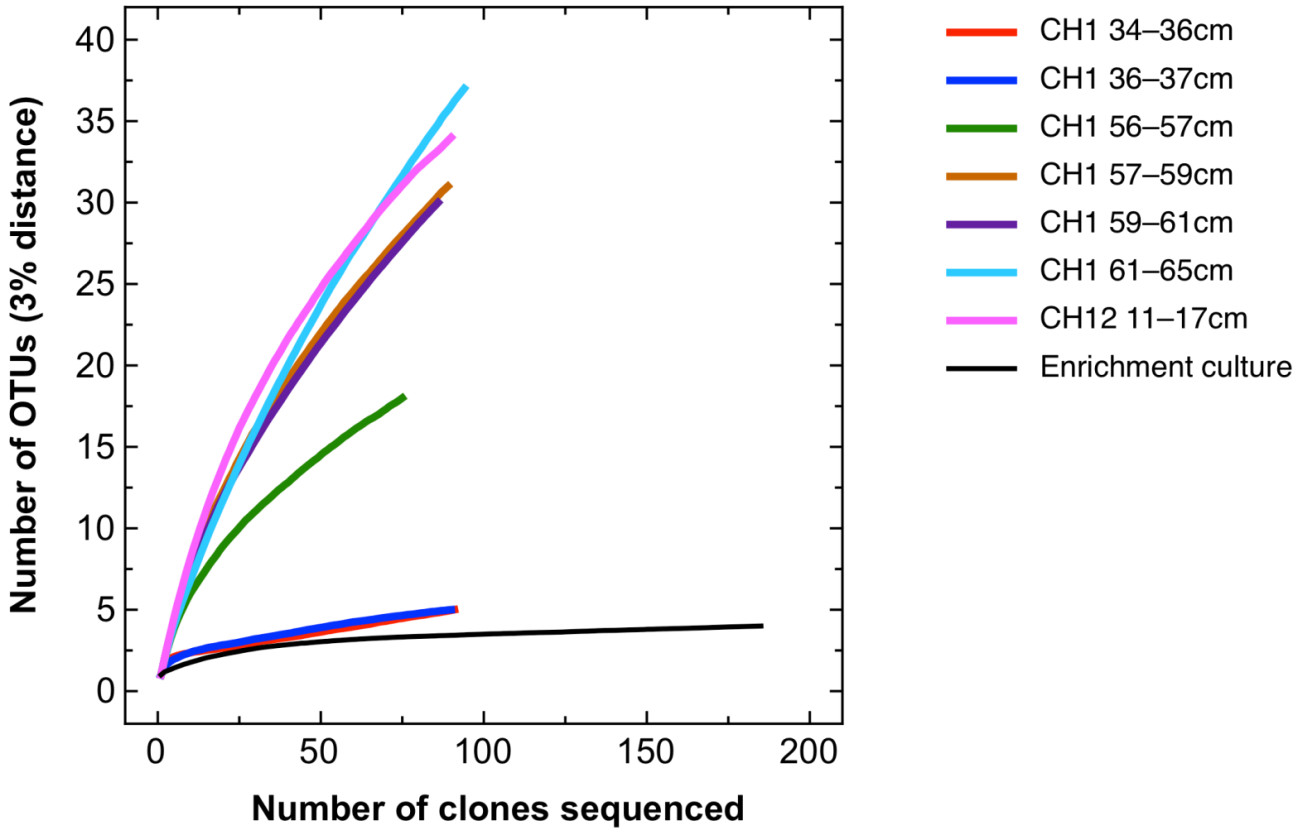
3.4. Supplmentry information



Supplementary Figure S1. Map of the multiple extreme, sulfidic mine tailings dump at Chañaral, Atacama Desert, northern Chile. The location of sampling sites for this study is marked with circles (modified after Dold, 2006, ref. 1).



Supplementary Figure S2. Detection of living bacterial cells in selected samples from the multiple extreme sulfidic mine tailings dump at Chañaral. Left: CARD-FISH probes EUB338 for the domain *Bacteria*, right: DAPI staining of total cells. Top: CH14, 25-26 cm; middle: CH14, 48-54 cm; bottom: CH12, 24-30 cm.



Supplementary Figure S3. Rarefaction curves for bacterial 16S rRNA gene sequences obtained from different depths of two sites of the multiple extreme sulfidic mine tailings dump at Chañaral, and from the Fe(II)-oxidizing enrichment culture (Fig. 2).

Supplementary Table S1. Mineral composition based on mineral liberation analysis of samples from different depths of the samling sites CH 1 and CH 12. Values in % vol. ND indicates not determined due to detection limit. Fe-oxide also includes Fe-hydroxide.

Sampling site	Depth (cm)	Pyrite	Fe-oxide	Halite	Calcite	Rutile	Quartz	Sulfate	Silicate	Others
CH 1	0-3	0.2	0.3	4.2	ND	0.4	45.2	2.7	45.7	1.1
	3-6	0.4	0.1	1.2	ND	0.3	56.1	3.9	37.5	0.6
	6-12	0.5	0.2	ND	ND	0.3	55.4	1.8	41.1	0.7
	12-22	ND	0.1	ND	ND	0.3	56.8	1	41.3	0.5
	22-27	ND	0.2	ND	ND	0.4	56.8	1.6	40.4	0.7
	27-33	ND	0.1	ND	ND	0.3	56.9	0.8	41.4	0.5
	34-36	0.2	0.2	ND	ND	0.5	56.3	4.7	37.3	0.8
	36-37	1.8	0.2	ND	ND	0.5	55.8	1.7	39.1	0.9
	37-40	0.1	0.1	ND	ND	0.3	59.6	1.7	37.6	0.6
	40-43	0.6	0.2	ND	ND	0.3	60.7	1.7	36	0.6
	43-45	0.7	0.2	ND	ND	0.4	60.5	1.5	35.9	0.8
	45-54	2.7	0.4	ND	ND	0.8	49.6	1.7	43.5	1.3
	56-57	1.1	0.4	ND	0.3	0.5	57.4	1.3	37.9	1
	57-59	1	1.7	ND	0.2	0.6	49.5	1.1	44.2	1.9
	59-61	0.2	1.4	ND	2.9	0.1	29.9	0.8	63.6	1.1
	61-65	0.1	1.9	ND	4.1	0.1	16.4	2	74.1	1.5
CH 12	11-17	0.7	2.3	ND	ND	0.8	48.4	1.6	44.6	1.6
	24-30	ND	2.2	ND	ND	1.4	54.6	2.5	37.5	1.8
	35-41	1.1	1.5	ND	ND	0.5	48.2	1.5	45.9	1.2
	45-52	0.6	0.6	ND	ND	0.4	46	1.3	50.1	1.2
	65-71	6	3.4	ND	ND	1.5	3.2	3.7	77.6	4.5
	74-80	6.3	3.4	ND	ND	1.7	51.9	2.9	29.8	3.9
	84-90	2.3	2.8	ND	ND	0.7	54.3	2.2	36.1	1.7
	100-105	0.1	0.8	ND	ND	0.5	55.9	1.6	40	1.1

Supplementary Table S2. Total elemental composition of the samples from five sites of the multiple extreme sulfidic mine tailings dump at Chañaral. The content of total carbon (TC), total organic carbon (TOC), total sulfur (TS) and other elements is given in weight percent.

Sampling site	Depth (cm)	Si	Al	Fe	Mn	Mg	Ca	Na	K	Cu	Zn	TC	TOC	TS
CH 1	0-3	12.2	3.5	1.1	0.01	0.6	0.5	16.8	1.7	0.1	0.0005	<0.01	<0.01	1.8
	3-6	25.6	8.1	1.8	0.01	0.7	0.9	3.6	2.4	0.2	0.002	<0.01	<0.01	2
	6-12	29.3	7.5	1.8	0.009	0.5	0.4	2.8	2.3	0.2	0.002	<0.01	<0.01	1.6
	12-22	32.6	7.3	1.3	0.005	0.3	0.4	1.8	2.3	0.09	0.002	<0.01	<0.01	0.9
	22-27	31.8	7.8	1.4	0.005	0.3	0.3	2	2.4	0.1	0.002	<0.01	<0.01	0.8
	27-33	33.3	7.9	1.2	0.004	0.3	0.4	1.2	2.5	0.04	0.001	<0.01	<0.01	0.6
	34-36	27.6	10.4	2.6	0.005	0.4	0.7	1.2	2.8	0.07	0.002	<0.01	<0.01	1.3
	36-37	27.3	11.4	2.2	0.004	0.4	0.3	1.2	3	0.04	0.002	<0.01	<0.01	1.8
	37-40	33	7.6	1.7	0.004	0.3	0.5	1.1	2.3	0.05	0.001	<0.01	<0.01	0.8
	40-43	33	7.7	1.6	0.004	0.3	0.5	1.1	2.3	0.04	0.001	<0.01	<0.01	1.5
	43-45	32.3	8.2	1.7	0.004	0.3	0.4	1.1	2.4	0.08	0.002	<0.01	<0.01	1.3
	45-54	31.7	8.2	2.2	0.005	0.3	0.3	0.9	2.4	0.2	0.002	<0.01	<0.01	1.9
	56-57	26.8	11.5	2.8	0.01	0.5	0.5	1	2.9	0.3	0.005	0.08	0.02	1.3
	57-59	31.8	7.7	2.8	0.02	0.6	0.8	1.3	2.4	0.3	0.004	0.06	<0.01	0.9
	59-61	27.4	8	3.9	0.07	1.3	3.1	2	2	0.1	0.008	0.5	0.1	0.4
	61-65	26.9	8	3.8	0.06	1.3	3.5	1.9	1.9	0.04	0.007	0.6	0.1	0.7
CH 11	0-3	18.4	5.1	1.9	0.01	0.4	1.5	11.5	1.6	0.3	0.0004	<0.01	<0.01	2.6
	4-7	18.4	5.1	1.9	0.01	0.4	1.5	11.2	1.9	0.3	0.0006	<0.01	<0.01	2.6
	12-15	25.2	11	2.6	0.006	0.5	0.4	2.6	3	0.1	0.003	<0.01	<0.01	1.4
	20-25	23.1	12.8	2.5	0.006	0.6	0.2	2.7	3.3	0.1	0.003	<0.01	<0.01	1.3
	25-30	31.2	7.3	4	0.006	0.4	0.6	0.9	2.4	0.04	0.002	<0.01	<0.01	2.5
	34-36	25.4	11.2	3	0.004	0.5	0.5	2	3	0.07	0.002	<0.01	<0.01	1.3
	41-45	30.5	9	1.9	0.003	0.4	0.3	1.5	2.6	0.05	0.001	<0.01	<0.01	0.8
	53-55	24.8	8.5	3.1	0.009	0.4	3.3	1.4	2.2	0.05	0.002	<0.01	<0.01	3.2
	57-63	25.1	12.9	1.9	0.002	0.5	0.2	1.8	3.2	0.1	0.002	<0.01	<0.01	1.5
	63-65	25.9	11.7	3	0.005	0.5	0.3	2	2.7	0.06	0.003	<0.01	<0.01	0.5
	65-67	32.7	6.7	1.9	0.01	0.5	1.4	1.2	2.1	0.6	0.003	<0.01	<0.01	1
	67-70	24.5	12.9	1.6	0.01	0.6	0.3	2.1	3.4	0.5	0.005	<0.01	<0.01	0.6
	70-76	24.7	12.8	1.8	0.009	0.6	0.4	2	3.1	0.3	0.004	<0.01	<0.01	1
	100-105	31.2	6.2	4.2	0.03	0.6	1.6	1.6	2	1.1	0.01	<0.01	<0.01	1
CH 12	11-17	32.5	7.5	3.4	0.007	0.4	0.3	0.8	2.4	0.1	0.002	<0.01	<0.01	0.9
	24-30	32.2	6.9	3.8	0.004	0.3	0.2	0.8	2.4	0.03	0.001	<0.01	<0.01	0.9
	35-41	32.4	7.8	2.7	0.005	0.4	0.3	0.9	2.6	0.04	0.001	<0.01	<0.01	1.1
	45-52	32.8	8	2.2	0.004	0.4	0.3	1	2.6	0.04	0.002	<0.01	<0.01	0.9
	65-71	31.1	7	5	0.005	0.4	0.3	0.9	2.3	0.05	0.002	<0.01	<0.01	2.6
	74-80	26.3	5.9	11	0.005	0.3	0.2	0.9	2	0.04	0.002	<0.01	<0.01	7.2
	84-90	30.8	6.9	5.3	0.003	0.3	0.2	0.9	2.3	0.04	0.001	<0.01	<0.01	2.7
	100-105	33.1	7.1	2.7	0.002	0.3	0.2	1.1	2.3	0.04	0.001	<0.01	<0.01	0.9
CH 14	0-5	33.9	7.6	1.5	0.006	0.5	0.4	1.2	2.4	0.04	0.002	<0.01	<0.01	0.4
	5-7	24.7	5.7	13.3	0.03	0.5	1.3	0.7	1.8	0.07	0.005	<0.01	<0.01	2.7
	7-15	33.8	7.4	2	0.007	0.5	0.4	1	2.3	0.03	0.003	<0.01	<0.01	0.4
	17-25	33	7.1	2.9	0.006	0.5	0.4	1.1	2.2	0.04	0.003	<0.01	<0.01	0.5
	25-26	30.1	6.4	5.9	0.01	0.5	0.8	0.9	2.1	0.05	0.003	<0.01	<0.01	1
	27-30	33.8	7.3	1.9	0.005	0.5	0.4	1.1	2.3	0.03	0.002	<0.01	<0.01	0.4
	30-33	34	7.6	1.3	0.005	0.5	0.4	1.2	2.3	0.03	0.002	<0.01	<0.01	0.4
	33-38	33.9	7.3	1.8	0.005	0.5	0.4	1.1	2.2	0.03	0.002	<0.01	<0.01	0.4
	38-40	33.5	8.1	1.2	0.005	0.5	0.4	1.4	2.4	0.04	0.002	<0.01	<0.01	0.3
	48-54	33.9	7.7	1.2	0.005	0.5	0.3	1.3	2.4	0.3	0.002	<0.01	<0.01	0.3
	63-69	34.1	7	1.7	0.007	0.5	0.4	1.2	2.2	0.04	0.002	<0.01	<0.01	0.4
	77-83	34.1	7.3	1.2	0.005	0.5	0.5	1.3	2.2	0.2	0.002	<0.01	<0.01	0.6

Supplementary Table S3. Inventory of OTUs of isolated 16S rRNA gene sequences from selected samples from the multiple extreme sulfidic mine tailings dump at Chañaral.

OTU	Nr. of Sequences	Enrichment culture	CH1 (34-36 cm)	CH1 (36-37 cm)	CH1 (56-57 cm)	CH1 (57-59cm)	CH1 (59-61cm)	CH1 (61-65 cm)	CH12 (11-17 cm)
001	69		46	23					
002	168	168							
003	116	11	41	64					
004	29				18	9	2		
005	26				7		19		
006	26				19	6	1		
007	27				4	23			
008	11				9		2		
009	2				1	1			
010	4				2	2			
011	46		1				12	33	
012	5								5
013	10				3		7		
014	5								5
015	11				1	6	4		
016	11								11
017	5						2	3	
018	2							2	
019	2				1		1		
020	3								3
021	2					2			
022	3					1	2		
023	2							2	
024	2								2
025	9				3	4			2
026	3								3
027	5					5			
028	3						3		
029	2						1	1	
030	2					1	1		
031	1							1	
032	13								13
033	1							1	
034	5								5
035	12						1	11	
036	1					1			
037	2							2	
038	2			2					
039	1								1
040	3					1	2		
041	3							3	
042	9				1		8		
043	2								2
044	2							2	
045	5								5
046	1			1					
047	8						4	4	
048	3								3
049	1							1	
050	1								1
051	6	6							
052	1				1				
053	1							1	
054	1						1		
055	1							1	
056	1								1
057	1			1					
058	1						1		
059	1							1	

060	2					2
061	1				1	
062	1					1
063	1					1
064	1	1				
065	2		2			
066	2			1	1	
067	1					1
068	2		1	1		
069	6			3	3	
070	1					1
071	1		1			
072	1					1
073	1				1	
074	6					6
075	1				1	
076	1					1
077	1					1
078	1		1			
079	1		1			
080	2				2	
081	1					1
082	2		2			
083	1				1	
084	1			1		
085	1				1	
086	2					2
087	1		1			
088	1				1	
089	1	1				
090	1				1	
091	1				1	
092	1			1		
093	2					2
094	2		2			
095	2					2
096	1				1	
097	1		1			
098	1			1		
099	1				1	
100	1	1				
101	1			1		
102	1				1	
103	1					1
104	1		1			
105	1				1	
106	1				1	
107	1					1
108	1					1
109	1			1		
110	1					1
111	1					1
112	1				1	
113	1				1	
114	1		1			

Supplementary Table S4. Closest phylogenetic relative for the representative sequences of each OTU based on the constructed phylogenetic tree (Fig. 3). a) no information available, *not published.

OTU	Accession Number	Closest relative	Sampling source	Reference
001	DQ364428	Uncultured bacterium clone G13	Processed gold ore	*
002	HQ678140	Uncultured bacterium clone 1	Acidic saline drain	*
003	AY371272	*Sulfobacillus* sp. 4G	Estuary contaminated with mine tailings	*
004	JN038198	Uncultured *Actinomycetales* bacterium clone EK_CK647	Petroleum-contaminated soil, China	*
005	AM997318	Uncultured bacterium clone Ucm1520	South-Atlantic Ocean, Cape Basin	2
006	FJ205276	Uncultured gammaproteobacterium clone 22	Active hydrothermal field sediments, depth:1922m	*
007	HM598239	Uncultured bacterium clone SCS_HX36_4	Continental marginal slope of Xisha Trough, China Sea	*
008	HQ857608	Uncultured gammaproteobacterium clone BPS_CK90	Hydrocarbon contaminated saline alkaline soils	*
009	HQ190528	Uncultured bacterium clone BP51	Zhongyuan oil field	*
010	EU491359	Uncultured bacterium clone P0X3b5G05	Seafloor lavas from Hawai'i South Point X3	3
011	EF612382	Uncultured *Sulfobacillus* sp. clone K6-C156	Mine tailings, Klondyke Mill Site, Arizona, USA	4
012	EF117909	*Thiohalomonas denitrificans* strain HLD 2	Hypersaline lake sediment	5
013	HQ857608	Uncultured gammaproteobacterium clone BPS_CK90	Hydrocarbon contaminated saline alkaline soils	*
014	EU010162	Uncultured marine bacterium clone A9	Seawater	*
015	EU937837	Uncultured bacterium clone 3BR-1G	Riparian iron oxidizing biofilm	*
016	JF344548	Uncultured gammaproteobacterium clone TRAN-132	Hydrocarbon polluted marine sediments from Figueiras beach	6
017	HQ441581	Uncultured bacterium clone USP_CQ16	Root canal in primary endodontic infection	*
018	DQ234642	Uncultured bacterium clone DR546BH1103001SAD18	Subsurface water	*
019	HQ910307	Uncultured bacterium clone P-11_B8	Desert soil	*
020	HM237288	*Thalassomonas* sp. clone M-M1	Marine sand	*
021	JQ825121	Uncultured bacterium clone SN235	Soil	*
022	FJ203051	Uncultured bacterium clone SHFG434	*Montastraea faveolata* diseased tissues	7
023	HQ184001	Uncultured bacterium clone De20	Leachate sediment	8
024	DQ088766	Uncultured bacterium clone BE325FW032701CTS_hole1-20	Continental crust	9
025	DQ499322	Uncultured bacterium clone CV92	Microbial biofilm	10
026	JQ979027	Uncultured bacterium clone R-49	Agricultural soil with atrazine	*
027	GQ249487	Uncultured bacterium clone A23	Surface marine sediment	*
028	AB696546	Uncultured bacterium clone T10CLN17	Soil	*
029	HQ864006	Uncultured bacterium clone TP-DE-B45	Soil	*
030	AM882518	Uncultured bacterium clone 37	Oil polluted sediment	11
031	EU016414	Uncultured bacterium clone HS07Ba08	Benzene-degrading, iron-reducing enrichment	12
032	M90662	*Thiobacillus hydrothermalis*	Deep-sea hydrothermal vent	13
033	FR744605	Uncultured bacterium clone PWB003	Production water from the Halfdan oil field	14
034	DQ002871	*Gramella portivictoriae* strain UST040801-001	Marine sediment	15
035	FN397996	*Bacteroidetes* bacterium G13a-B	Gas hydrate containing sediment, Indian Ocean	16

036	JX222378	Uncultured bacterium clone EMIRGE_OTU_s2b2b_3090	Subsurface aquifer sediment	*
037	EU651877	Uncultured bacterium clone BiphS1_24	Biphenyl-degrading sulfate-reducing enrichment culture	17
038	EU443097	*Alcaligenes* sp. F78	a)	*
039	HQ912788	Uncultured *Pseudoxanthomonas* sp. clone ASC81	*Asparagus* straw compost	*
040	JF417942	Uncultured bacterium clone 1-2B-23	Dry anaerobic digester	18
041	HQ697807	Uncultured bacterium clone Bms_CK325	Hydrocarbon contaminated saline alkaline soil	*
042	HQ405627	Bacterium enrichment culture clone AOM-SR-B17	Enrichment anaerobic oxidation of methane	19
043	AB479193	*Pullulanibacillus* sp. D' C-1	Japan soil	*
044	HQ727572	Uncultured bacterium clone BC_COM515	Petroleum contaminated soil	*
045	AB013974	*Thiomicrospira frisia* strain JB-A2	DSM 12351	20
046	JF905606	*Brevibacterium* sp. 34	Soil 450	*
047	GU339478	Firmicutes bacterium enrichment culture clone VNC1B071	Biphenyl-degrading sulfate-reducing enrichment culture.	17
048	CP000109	*Thiomicrospira crunogena* XCL-2	a)	*
049	EU016416	Uncultured bacterium clone HS07Ba10	Benzene-degrading iron-reducing enrichment	12
050	JQ579995	Uncultured bacterium clone FII-AN054	Sediments from Figueiras Beach	*
051	GU120607	Uncultured bacterium clone PL-C1_6_2_E02	Southwest part of Pitch Lake	*
052	AB637080	Uncultured bacterium clone 12TCLN154	Soil	*
053	AB637116	Uncultured bacterium clone 12TCLN190	Soil	*
054	AY330129	Uncultured *Clostridium* sp. clone AC007	Methanogenic bioreactor	21
055	JF417917	Uncultured bacterium clone 1-1B-26	Dry anaerobic digester	18
056	HM591356	Uncultured bacterium clone SW-July-96	Seawater reverse osmosis process	*
057	EU362182	*Microbacterium* sp. C9	Dune sand	*
058	HM066339	Uncultured bacterium clone EDW07B002_19	Texas state well #DX 68-23-617	*
059	JF417939	Uncultured bacterium clone 1-2B-19	Dry anaerobic digester	18
060	EU735631	Uncultured bacterium clone SC145	Oil contaminated soil in Jidong oilfield	22
061	AB637088	Uncultured bacterium clone 12TCLN162.	Soil	
062	AB278144	Uncultured bacterium clone Mafs-EB04	Mariana Trough	23
063	GU552681	*Gaetbulibacter marinus* strain KYW382	Sea water	24
064	HQ857655	Uncultured bacterium clone BPS_CK180	Hydrocarbon contaminated saline alkaline soils	*
065	AJ306780	Uncultured bacterium clone SHA-100	a)	25
066	HQ916622	Uncultured bacterium clone LGH02-B-085	Lei-Gong-Huo mud volcano	26
067	AJ609270	*Marinobacter sediminum* strain R65	Marine environment	27
068	JF417942	Uncultured bacterium clone 1-2B-23	Dry anaerobic digester	18
069	HQ441581	Uncultured bacterium clone USP_CQ16	Root canal in primary endodontic infection	*
070	HM630208	Uncultured bacterium clone NT4_C13	Intestinal tract	*
071	JQ716325	Uncultured bacterium clone BJGMM-U117	Soil	*
072	HQ119903	Uncultured bacterium isolate 1112842460703	Loamy sand from a tomato field	28
073	JN039009	Uncultured bacterium clone P-B289	Chongxi wetland soil	*
074	DQ481463	*Polaribacter dokdonensis* strain MED152	NW Mediterranean Sea surface water	29
075	DQ148942	*Desulfotomaculum arcticum* strain 15	Cold sediment off Svalbard	30
076	AB377223	Gamma proteobacterium NAMAF009	Sea water	*
077	HQ396972	Uncultured *Gramella* sp. clone HAHS13.84	Haloalkaline soil	*
078	GU118297	Uncultured bacterium clone Dstr_N21	Environmental samples	31
079	JN825605	Uncultured bacterium clone Alchichica_AQ1_1_1B_32	South-Atlantic Ocean,Cape Basin	2
080	AB630810	Uncultured bacterium clone MPB2-134	Aquatic moss pillars	32

081	AB530197	Uncultured bacterium clone Suez.16S.Bac.29	Marine sediment	33
082	JQ800897	Uncultured bacterium clone BJGMM-3s-97	Soil	*
083	AB260050	*Halanaerobiales* bacterium Ag-C55	Black rust from borehole	34
084	AF477014	*Balamuthia mandrillaris* isolate V194	Isolate V194	35
085	EU735628	Uncultured bacterium clone SC137	Mariana Trough	23
086	FM242243	Uncultured bacterium clone 94 T0h-oil	Environmental sample	36
087	HM128252	Uncultured bacterium clone SINH778	Environmental sample	*
088	HM635205	*Clostridia* enrichment culture clone WSC-8	Soil	*
089	JQ579946	Uncultured bacterium clone FII-AN005	Sediments from Figueiras Beach	*
090	AB637256	Uncultured bacterium clone 12TCLN330	Soil	*
091	HQ727572	Uncultured *Firmicutes* bacterium, clone BC_COM515	Petroleum contaminated soil	*
092	JQ800945	Uncultured bacterium clone BJGMM-3s-187	Soil	*
093	EU328041	Uncultured *Marinobacter* sp., clone B158	Moderate saline soil	*
094	AM997511	Uncultured deep-sea bacterium clone UncDeep9	Southern Atlantic Ocean, Cape Basin	2
095	HM598193	Uncultured bacterium clone SCS_HX28_140	Surface sediment, Xisha Trough, China Sea	*
096	JN536244	Uncultured organism clone SBZP_1931	Guerrero Negro Hypersaline Mat	*
097	FN594690	Uncultured *Planctomycetaceae* clone a4-2	Biofilom from gold mine in Zloty Stok	*
098	HQ697916	*Fontibacillus* sp. enrichment culture clone DF60	Offshore mangrove sediment	37
099	GU455264	Uncultured bacterium clone thermophilic alkaline-24	Activated sludge fermentation reactor	38
100	FN553640	Uncultured sediment bacterium clone 285-65	Logatchev hydrothermal vent field	*
101	HQ697794	Uncultured bacterium clone Bms_CK298	Hydrocarbon contaminated saline alkaline soil	*
102	JF417918	Uncultured bacterium clone 1-1B-27	Dry anaerobic digester	18
103	HQ397469	Uncultured bacterium clone HSS123	Saline soil from Gujarat coast	*
104	FJ543076	Uncultured actinobacterium clone B10-05C	High ergovaline treatment gut	39
105	HQ727572	Uncultured *Firmicutes* bacterium clone BC_COM515	Petroleum contaminated soil	*
106	AB534039	Uncultured bacterium clone 75-P1	Salt pan	*
107	DQ015780	Uncultured bacterium clone ELB25-004	Lake water, East Lobe, 25 m	40
108	EU369165	Uncultured bacterium clone MBFOS-06	Oyster shell	*
109	HE604746	Uncultured bacterium clone Kasin-B3-F04	Hypersaline lake sediment	41
110	HQ396970	Uncultured *Gramella* sp., clone HAHS13.80	Haloalkaline soil	*
111	HQ857678	Uncultured *Salinimicrobium* sp., clone BPS_H498	Hydrocarbon contaminated saline alkaline soil	*
112	HQ697807	Uncultured bacterium clone Bms_CK325	Hydrocarbon contaminated saline alkaline soil	*
113	JX133331	*Bacillus mannanilyticus* 12TCLN195	Soil	*
114	HQ912786	Uncultured bacterium clone ASC44	*Asparagus* straw compost	*

References

1. Unpublished
2. Couradeau, E. et *al*. Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline Lake Alchichica (Mexico). *PloS One* 6, 1-16 (2011).
3. Schauer, R., Bienhold, C., Ramette, A. & Harder, J. Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. *ISME J.* 4, 159-170 (2010).
4. Santelli, C. M. *et al.* Abundance and diversity of microbial life in ocean crust. *Nature* 453, 653-657 (2008).

5. Donachie, S. P. *et al.* The Hawaiian archipelago: A microbial diversity hotspot. *Microb. Ecol.* 48, 509-520 (2004).
6. Schauer, R. *et al*. Bacterial sulfur cycling shapes microbial communities in surface sediments of an ultramafic hydrothermal vent field. *Environ. Microbiol*. 13, 2633-2648 (2011).
7. Sunagawa, S. *et al.* Bacterial diversity and White Plague Disease - associated community changes in the Caribbean coral Montastraea faveolata. *ISME J*. 3, 512-521 (2009).
8. Liu, J., Wu, W., Chen, C., Sun, F. & Chen, Y. Prokaryotic diversity, composition structure, and phylogenetic analysis of microbial communities in leachate sediment ecosystems. *Appl. Environ. Microbiol*. 91, 1659-1675 (2011).
9. Lin, L. *et al.* Planktonic microbial communities associated with fracture-derived groundwater in a deep gold mine of South Africa. *Geomicrobiol. J.* 23, 475-497 (2006).
10. Paissé, S. *et al.* Structure of bacterial communities along a hydrocarbon contamination gradient in a coastal sediment. *FEMS Microbiol. Ecol*. 66, 295-305 (2008).
11. Kunapuli, U., Lueders, T. & Meckenstock, R. U. The use of stable isotope probing to identify key iron-reducing microorganisms involved in anaerobic benzene degradation. *ISME J.* 1, 643-653 (2007).
12. Gittel, A., Kofoed, M., Sørensen, K. B., Ingvorsen, K. Schramm, A. Succession of Deferribacteres and Epsilonproteobacteria through a nitrate-treated high-temperature oil production facility. *Syst. Appl. Microbiol*. 35, 165-174 (2012).
13. Bauer, M. *et al*., Whole genome analysis of the marine Bacteroidetes *'Gramella forsetii*' reveals adaptations to degradation of polymeric organic matter. *Environ. Microbiol*. 8, 2201-2213 (2006).
14. Parkes, R. J. *et al.* Culturable prokaryotic diversity of deep, gas hydrate sediments: first use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG). *Environ. Microbiol.* 11, 3140-3153 (2009).
15. Shimizu, S. *et al.* Molecular characterization of microbial communities in fault-bordered aquifers in the Miocene formation of northernmost Japan. *Geobiol.* 4, 147-223 (2006).
16. Tang, Y. Q. *et al.* Characteristic microbial community of a dry thermophilic methanogenic digester: its long-term stability and change with feeding. *Appl. Microbiol. Biotechnol.* 91, 1447-1461 (2011).
17. Zhang, Y., Maignien, L., Zhao, X., Wang, F. & Boon, N. Enrichment of a microbial community performing anaerobic oxidation of methane in a continuous high-pressure bioreactor. *BMC. Microbiol.* 11, 137 (2011).
18. Brinkhoff, T. & Muyzer, G. Increased species diversity and extended habitat range of sulfur-oxidizing *Thiomicrospira* spp. *Appl. Environ. Microbiol*. 63, 3789-3796 (1997).
19. Burrell, P.C., O'Sullivan, C., Song, H., Clarke, W. P. & Blackall, L. L. Identification, detection, and spatial resolution of *Clostridium* populations responsible for cellulose degradation in methanogenic landfill leachate bioreactor. *Appl. Environ. Microbiol*. 70, 2414-2419 (2004).
20. Liu, R. *et al.* Comparison of archaeal and bacterial community structures in heavily oil-contaminated and pristine soils. *J. Biosci. Bioeng*. 108, 400-407 (2009).

21. Naganuma, T., Miyoshi, T. & Kimura, H. Phylotype diversity of deep-sea hydrothermal vent prokaryotes trapped by 0.2- and 0.1-µm-pore-size filters. *Extremophiles* 11, 637-646 (2007).
22. Park, S. C., Choe, H. N., Baik, K. S., Lee, K. H. & Seong, C. N. *Gaetbulibacter aestuarii* sp. nov., isolated from shallow coastal seawater, and emended description of the genus *Gaetbulibacter*. *Int. J. Syst. Evol. Microbiol*. 62, 150-154 (2012).
23. Schlöteöburg, C. *Mikrobielle Diversität und Dynamik einer 1,2-Dichlorpropan dechlorierenden Mischkultur* Thesis, Humbold University, Berlin (2001).
24. Chang, Y. H. Microbial methane cycling in a terrestrial mud volcano in eastern Taiwan. *Environ. Microbiol.* 14, 895-908 (2012).
25. Romanenko, L. A. *et al. Marinobacter bryozoorum* sp. nov. and *Marinobacter sediminum* sp. nov., novel bacteria from the marine environment. *Int. J. Syst. Evol. Microbiol.* 55, 143-148 (2005).
26. Sylvan, J. B., Toner, B. M. & Edwards, K. J. Life and death of deep-sea vents: bacterial diversity and ecosystem succession on inactive hydrothermal sulfides. *MBio.* 3, 1-10 (2012).
27. Williamson, K. E., Kan, J., Polson, S. W. & Williamson, S. J. Optimizing the indirect extraction of prokaryotic DNA from soils. *Soil Biol. Biochem*. 43, 736-748 (2011).
28. Gomez-Consarnau, L. *et al.* Light stimulates growth of proteorhodopsin-containing marine Flavobacteria. *Nature* 445, 210-213 (2007).
29. Vandieken, V., Knoblauch, C. & Jorgensen, B. B. *Desulfotomaculum arcticum* sp. nov., a novel spore-forming, moderately thermophilic, sulfate-reducing bacterium isolated from a ermanently cold fjord sediment of Svalbard. *Int. J. Syst. Evol. Microbiol*. 56, 687-690 (2006).
30. Sunagawa, S., Woodley, C. M. & Medina, M. Threatened corals provide underexplored microbial habitats. *PLoS ONE* 5, E9554 (2010).
31. Nakai, R. *et al.* Microflorae of aquatic moss pillars in a freshwater lake, East Antarctica, based on fatty acid and 16S rRNA gene analyses. *Polar Biol.* 35, 425-433 (2012).
32. Elsaied, H. *et al.* Marine integrons containing novel integrase genes, attachment sites, *attI*, and associated gene cassettes in polluted sediments from Suez and Tokyo Bays. *ISME J.* 5, 1162-1177 (2011).
33. Nakagawa, S. *et al.* Microbial community in black rust exposed to hot ridge flank crustal fluids *Appl. Environ. Microbiol.* 72, 6789-6799 (2006).
34. Booton, G. C., Carmichael, J. R., Visvesvara, G. S., Byers, T. J. & Fuerst, P. A. Identification of *Balamuthia mandrillaris* by PCR assay using the mitochondrial 16S rRNA gene as a target. *J.Clin. Microbiol*. 41, 453-455 (2003).
35. Paisse, S., Goni-Urriza, M., Coulon, F. & Duran, R. How a bacterial community originating from a contaminated coastal sediment responds to an oil input. *Microb. Ecol.* 60, 394-405 (2010).
36. Zhao, C. & Ruan, L. Biodegradation of *Enteromorpha prolifera* by mangrove degrading micro community with physical-chemical pretreatment. *Appl. Microbiol. Biotechnol.* 92, 709-716 (2011).
37. Zhang, P. *et al.* Understanding short-chain fatty acids accumulation enhanced in waste activated sludge alkaline fermentation: kinetics and microbiology. *Environ. Sci. Technol*. 44, 9343-9348 (2010).

38. Rattray, R. M., Perumbakkam, S., Smith, F. & Craig, A. M. Microbiomic comparison of the intestine of the earthworm *Eisenia fetida* fed ergovaline. *Curr. Microbiol.* 60, 229-235 (2010).
39. Glatz, R. E., Lepp, P. W., Ward, B. B. & Francis, C. A. Planktonic microbial community composition across steep physical/chemical gradients in permanently ice-covered Lake Bonney, Antarctica. *Geobiol.* 4, 53-67 (2006).
40. Emmerich, M. *et al.* Aundance, distribution, and activity of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in hypersaline sediments of lake Kasin, Southern Russia. *Appl. Environ. Microbiol*. 78, 4386-4399 (2012).

Supplementary Table S5. Richness estimators and diversity indices for *Bacteria* calculated using MOTHUR v1.29 compared at a distance level of 0.03.

Group	N_{seqs} [a]	S_{obs} [b]	ACE [c]	Chao1	Coverage	Shannon	Simpson
Chanaral12 11-17cm	87	35	80	54	79%	3.21	0.04
Chanaral1 34-36cm	90	4	NP [d]	5	98%	0.80	0.47
Chanaral1 36-37cm	90	5	8	6	98%	0.78	0.55
Chanaral1 56-57cm	85	23	40	38	88%	2.69	0.09
Chanaral1 57-59cm	91	32	108	64	78%	2.84	0.09
Chanaral1 59-61cm	86	30	125	73	78%	2.82	0.09
Chanaral1 61-65cm	86	34	82	85	73%	2.80	0.12
Enrichment culture	185	4	5	4	99%	0.38	0.83

[a] Number of sequences
[b] Number of Operational Taxonomic Units (OTU)
[c] Abundance-based Coverage Estimator
[d] Not possible to calculate

Chapter 4

Microbial diversity at the moderate acidic stage in three different sulfidic mine tailings dumps generating acid mine drainage (Research in Microbiology 165 (2014) 713-718.)

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Abstract

In freshly deposited sulfidic mine tailings the pH is alkaline or circumneutral. Due to pyrite or pyrrhotite oxidation the pH is dropping over time to pH values <3 at which acidophilic iron- and sulfur-oxidizing prokaryotes prevail and accelerate the oxidation processes, well described for several mine waste sites. The microbial communities at the moderate acidic stage in mine tailings are only scarcely studied. Here we investigated the microbial diversity via 16S rRNA gene sequence analysis in eight samples (pH range 3.2-6.5) from three different sulfidic mine tailings dumps in Botswana, Germany and Sweden. In total 701 partial 16S rRNA gene sequences revealed a divergent microbial community between the three sites and at different tailings depths. *Proteobacteria* and *Firmicutes* were overall the most abundant phyla in the clone libraries. *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Nitrospira* occurred less frequently. The found microbial communities were completely different to microbial communities in tailings at <pH 3 described in the literature.

4.1. Introduction

Mine tailings are fine-grained waste left over from the metal extraction process. This material often contains several percent of metal sulfides, i.e. pyrite (FeS_2) or pyrrhotite ($Fe_{1-x}S$, with x = 0-0.125). In freshly deposited sulfidic mine tailings the pH is alkaline or circumneutral. Due to exposing the tailings material to air and water, the metal sulfides get oxidized and the pH is dropping over time to low values, and acid mine drainage is generated. Over time an acidic oxidized zone enriched in secondary minerals such as iron(III)hydroxides develops above an unoxidized zone with unaltered material in tailings dumps [1-4].

The metal sulfide oxidation process is dramatically accelerated by iron- and sulfur-oxidizing prokaryotes at pH values below 3, at which ferric iron remains in solution and can serve as efficient oxidant for the metal sulfides, thereby being reduced to ferrous iron. The ferrous iron is oxidized to ferric iron by acidophilic iron-oxidizers such as *Acidithiobacillus* spp., *Leptosprillum* spp., *Ferroplasma* spp., '*Ferrovum'* spp. *Alicyclobacillus* spp. and *Sulfobacillus* spp. [5,6]. Acidophilic iron-oxidizers are regularly found in acid mine drainage [7-11], in bioleaching operations [6,12], in acidic abandoned mines [13,14] as well as in mine tailings at low pH [4].

The microbial diversity in several sulfidic mine tailings has been studied based on cultivation approaches [1,15e21] as well as by 16S rRNA gene sequencing [20,22e29]. Previous tailings studies revealed the predominance of iron- and sulfur-oxidizing acidophiles at low pH, but microbial communities at the moderately acidic oxidation stage (between the initial circumneutral to alkaline pH and the strong acidic final stage) have only been studied for mine tailings sites in China [24,26-28].

For further exploration of these communities at different geochemical tailings properties and climatic conditions we investigated the microbial diversity via 16S rRNA gene sequence analysis in eight samples in the pH range 3.2-6.5 from three different sulfidic mine tailings dumps located in Botswana, Germany and Sweden. Quantitative microbial community analysis of the same acid mine drainage generating tailings focused previously on the acidophiles *Acidithiobacillus* spp., *Leptosprillum* spp. and *Sulfobacillus* spp. [1].

4.2. Materials and methods

4.2.1. Field site description

Samples from different tailings depths were collected between 2003 and 2006 from three sulfidic mine tailings dumps near Selebi-Phikwe (Botswana), Freiberg (Germany), and Kristineberg (Sweden), as described previously [1,19,21,30,31]. The uncovered and active tailings dump near Selebi-Phikwe contained the reactive pyrrhotite as the main metal sulfide. Alternating grey and brown layers were found throughout the whole tailings depth profile. The brown color originated from precipitation of iron(III)hydroxides. The climate is semi-arid with an average annual temperature of 21 ˚C [30]. The climate at the covered pyrite-containing tailings dump at Kristineberg is cold and humid with an annual average temperature of 0.7 ˚C. Before covering in 1996 with about 2 m thick till, pyrite oxidation led to a pronounced about 0.5 m thick brown oxidized zone [31]. The uncovered and inactive tailings located in Freiberg at temperate climate with an annual average temperature of 7.7 ˚C contained as main metal sulfides pyrite, arsenopyrite, sphalerite, and galena. Due to vertical metal transport in the oxidized zone and metal precipitation, a brown hardpan formed at a distinct depth within the tailings [19]. Eight samples from different sampling depths from the three tailings in the pH range 3.2-6.5 were selected for 16S rRNA gene cloning and sequencing. Geochemical and microbial data for the eight selected samples obtained in previous studies are presented in Table 1. The microbial community was dominated by *Bacteria*, the *Archaea* and *Eukarya* played only a minor role in all tailings [1]. Thus, our diversity analysis focused on *Bacteria*.

4.2.2. DNA extraction and 16S rRNA gene library construction

The extraction of genomic DNA was performed with 0.5 g of a frozen tailings sample using the FastDNA Spin Kit for soil (Bio 101) protocol. Community 16S rRNA genes were amplified by PCR with the universal bacterial primer pairs GM3f (50 AGA GTT TGATCATGG C 30) and GM4r (50 TAC CTT GTT ACG ACT T 30) [32] for *Bacteria* in 50 ml final volume. PCR mixture was prepared from Thermo Scientific MasterMix (final concentration: 75 mM Tris-HCL (pH 8.8), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 mM of each primer, 0.652 U ThermoPrime Tag DNA polymerase, 100 ng/mL BSA) and 2 mL template of extracted DNA. Negative controls without template were used as a contamination check. Amplification reactions occurred during 35 cycles and included denaturation at 95 ˚C for 45 s, extension at 72 ˚C for 90 s and annealing at 55 ˚C for 30 s. Afterwards the amplicons of PCR reactions were commercially cloned and sequenced by DNA Sanger sequencing, LGC Genomics, Berlin, Germany.

Overlapping sequencing from both sides of the 16S rRNA genes was performed. Contigs were constructed with the software Geneious Pro 5.4 and were aligned and checked for chimeric artifacts with UCHIME implemented in the Mothur v1.30 program package [33] and discarded if discovered. In total 701 partial 16S rRNA gene fragments were obtained after quality-, alignment-, and chimera-check for subsequent analysis. The obtained 16S rRNA gene sequences were assigned to 105 operational taxonomic units (OTU, 97% similarity) and representative sequences from each OTU were selected by the average distance criteria. The representative OTU sequences were taxonomically assigned using the SINA online aligner with the ARB sequences database SSURef_NR99_117 as template. For phylogenetic analysis the sequences alignment was curated by hand with the ARB software package (v.5.4) [34]. Sequences that could not be aligned reliable were discarded from the phylogenetic

tree construction. For phylogenetic tree construction selected reference sequences (ten for each OTU) together with the OTU-representatives were used for maximum likelihood algorithm (RAxML) with GTRGAMMA as rate distribution model [35] available from the CIPRES Science Gateway [36], the general bacteria filter provided in ARB, and a bootstrap test with 1000 replicates. The 16S rRNA gene sequence from Nitrosopumilus maritimus SCM1 (CP000866) was used as an out-group. The 16S rRNA gene sequences obtained in this study were submitted to GenBank with the accession numbers KJ650684-KJ650788.

4.3. Results and discussion

The microbial communities in three different sulfidic mine tailings dumps at pH 3.2 to 6.5 were identified by 16S rRNA gene sequencing analysis and the overall results revealed a strong differentiation of the microbial composition with depth as well as between the three tailings. On the one hand the microbial community composition shifts since the tailings dumps samples were in different acidic stages due to ongoing oxidation processes. On the other hand the activity of microorganisms altered the geochemical conditions of the tailings dump that resulted in a high abundance of acidophilic ironand sulfur-oxidizing chemolithotrophs at the final weathering stage [1]. In previous investigations the role of pH as most important factor for the microbial community composition and the geochemistry was determined in tailings field site studies [24,26-28] as well as in laboratory experiments [37,38]. The taxonomic classification of the obtained bacterial 16S rRNA gene sequences in this study clustered in the six phyla Proteobacteria, Firmicutes, Nitrospirae, Actinobacteria, Acidobacteria, and Bacteroidetes. The most abundant OTUs contained

representatives of Firmicutes and Proteobacteria (Table 2). The two samples with pH 6.4 (S1) and 6.5 (K1) at the uppermost sampling depth exhibited a much higher

Table 1. Geochemical and microbial data for the eight selected samples (clone libraries) from the different sulfide mine tailings dumps generating acid mine drainage; NA = not analyzed; ND= not detected; cell numbers are given in log N per gram dry weight; qPCR data are given in log 16S/18S rRNA gene or *dsr* copy numbers per gram dry weight (data from references 1, 19, 21, 30, 31).

Sample	S1	S2	S3	S4	K1	K2	K3	F1
Tailings location	Selebi-Phikwe (Botswana)				Kristineberg (Sweden)			Freiberg (Germany)
Climate	semi-arid				cold and humid			temperate
Metal sulfide	pyrrhotite				pyrite			pyrite, arsenopyrite sphalerite, galena
Depth (m)	0-1	6-7	9-10	10-11	3.6-3.75	4.8-4.9	4.9-5.0	1.3
Color	grey-brown	grey-brown	grey-brown	grey-brown	grey	brown	grey	brown (hardpan)
pH	6.4	3.4	3.2	3.5	6.5	4.6	5.3	3.6
C_{total} (%)	NA	NA	NA	NA	0.53	0.23	0.18	0.08
$C_{organic}$ (%)	0.03	0.03	0.04	0.05	NA	NA	NA	0.03
S_{total} (%)	5.5	3.5	1.6	2.5	18.1	0.66	17.1	1.7
Fe_{total} (%)	16.0	12.9	10.3	11.7	6.79	7.16	16.44	NA
Cu_{total} (mg/kg)	519	399	257	286	181	89	1777	NA
Total cell counts	7.4	7.6	7.9	7.6	7.7	8.5	6.4	8.1
MPN acidophilic Fe-oxidizers	2.2	5.5	7.1	6.7	2.2	4.6	ND	4.2
qPCR Prokaryotes	7.4	7.3	9.3	8.4	8.8	9.6	7.8	7.8
qPCR Bacteria	7.3	7.7	8.8	8.0	8.8	9.6	7.1	8.5
qPCR Archaea	ND	ND	4.9	ND	ND	7.0	ND	ND
qPCR Eukarya	ND	ND	4.5	ND	5.3	5.2	5.6	ND
qPCR *Acidithiobacillus*	ND	4.7	5.0	4.7	NA	NA	4.6	ND
qPCR *Leptospirillum*	ND	ND	4.8	ND	NA	NA	ND	6.4
qPCR *Sulfobacillus*	ND	ND	5.4	ND	NA	NA	ND	ND
qPCR *dsr*	ND	ND	6.0	ND	6.9	5.6	5.9	ND

diversity than all other samples at pH 3.2 to 5.3. This finding of a decreasing diversity with decreasing pH is in agreement with previous studies of the microbial diversity in mine tailings [24,28]. Chen et al. [27] revealed different results, but from the surface (upper 5 cm) of the investigated tailings. In Selebi-Phikwe, most of the OTUs were phylogenetically assigned to the physiologically diverse *Proteobacteria* (47%) and

Firmicutes (44%) at the tailings surface (S1) at pH 6.4. In the underneath layers (S2-S4, pH 3.2-3.5) *Proteobacteria* were almost not detected and the phylum *Firmicutes* was dominant. A similar pattern was observed for Kristineberg, where *Proteobacteria* (49%) and *Actinobacteria* (47%) were most abundant at the surface (K1) at pH 6.5, but absent in the two deeper samples (K2, K3, pH 4.6 and 5.3), where *Firmicutes* prevailed. *Actinobacteria* likely originated from the soil cover above this uppermost sample layer. *Proteobacteria* was also the dominant phylum in Freiberg (F1) with 97% sequence abundance. In contrast to the Selebi-Phikwe and Kristineberg samples, the phylum *Firmicutes* was not identified in the microbial community at Freiberg.

The high abundance of potentially spore-forming Grampositive bacteria (*Firmicutes* and *Actinobacteria*) in Selebi-Phikwe and Kristineberg suggests that a large part of the microbial community is metabolically inactive there. These two tailings were less densely colonized by bacteria than the one in Freiberg with the highest cell numbers and microbial pyriteeoxidation activity [1].

The relative abundance of representative bacterial families in the clone libraries of the eight samples is shown in Fig. 1.

The families *Alicyclobacillaceae* and *Peptococcaceae* (*Firmicutes*), *Hydrogenophilaceae* (*Proteobacteria*), and *Micrococcaceae* (*Actinobacteria*) were most abundant in the tailings dumps. *Alicyclobacillaceae* contain acidophilic iron- and sulfur-oxidizing microorganisms. Peptococcaceae comprise sulfate-reducing bacteria. Hydrogenophilaceae contain the sulfur-oxidizing genus Thiobacillus. The pattern showed a high variation between the samples. Most sequences for Selebi-Phikwe were related to Alicyclobacillaceae, Peptococcaceae and Hydrogenophilaceae. In Kristineberg the most abundant families were Alicyclobacillaceae, Hydrogenophilaceae, Peptococcaceae and Micrococcaceae. The clone library for

Freiberg showed a predominance of Hydrogenophilaceae. Phylogenetic trees for the Bacteria and particularly for the phylum Firmicutes of representative 16S rRNA gene sequences retrieved from the three different tailings dumps are shown as Supplementary Figs. S1 and S2. Sequences related to the sulfur-oxidizers Thiobacillus spp. and distantly related to the acidophilic iron-oxidizer Leptospirillum spp. were found. These organisms might be involved in metal sulfide oxidation. Sequences related to Arthrobacter spp. and other Actinobacteria were found for the Kristineberg site and are likely introduced from the soil cover. The Firmicutes related OTUs were e.g. affiliated to iron- and sulfur-oxidizing Alicyclobacillus spp., thiosulfate-reducing Moorella spp. and sulfatereducing Desulfurispora spp., Desulfitobacterium spp. and Desulfosporosinus spp. [39].

Table 2. 16S rRNA gene sequences divergence in the three different tailings dumps at phylum level.

Phylum	S1	S2	S3	S4	K1	K2	K3	F1
Acidobacteria	-	-	-	-	-	2	-	-
Actinobacteria	7	-	-	-	39	-	-	1
Bacteroidetes	-	-	-	-	-	1	-	-
Firmicutes	39	58	75	56	3	86	90	-
Nitrospirae	1	-	-	-	-	-	-	1
Proteobacteria	41	1	-	-	40	-	-	86
unclassified	-	24	12	31	3	3	-	1

Sulfate-reducers from the phyla Firmicutes (including *Desulfosporosinus* spp.) and *Proteobacteria* were previously found in other mine tailings [16,25,40] as well as in other sulfate-rich heavy metal contaminated environments [41,42]. Members of the genus *Desulfosporosinus* can also reduce ferric iron and grow under acidic conditions [39]. The sequences related to the genera *Paenibacillus* and *Bacillus* have been frequently detected in heavy metal contaminated soils [23,43]. Quantification of

sulfate-reducers via their functional gene dsr and qPCR revealed their high abundance in the tailings of Kristineberg (Table 1).

For comparison, the microbial community composition in other mine tailings is discussed. A Chinese research group [20,26,27] analyzed lead/zinc mine tailings communities in samples with a pH range 1.8-7.5. They found a predominance of *Proteobacteria* including the hydrogen and sulfur-oxidizing genera *Hydrogenophaga*, *Thiovirga* and *Thiobacillus*, respectively, in the circumneutral samples at the initial weathering stage. In contrast, gene sequences related to the acidophilic, iron-oxidizing genera *Ferroplasma* (*Euryarchaeota*), *Acidithiobacillus* (*Proteobacteria*), *Leptospirillum* (*Nitrospira*) and *Sulfobacillus* (*Firmicutes*) were mainly detected in the samples with low pH and more intense weathering and iron precipitations.

In addition *Actinobacteria* and *Bacteroidetes* were detected in all six samples. Analysis of the microbial community of copper mine tailings in China via 16S rRNA gene-targeted 454 pyrosequencing (28) revealed an increasing relative abundance of *Euryarchaeota* and *Firmicutes* and a decreasing abundance of *Actinobacteria* and all classes of *Proteobacteria* except *Gammaproteobacteria* with decreasing pH, reflecting the ongoing oxidation progress in the tailings. The composition of the microbial community was correlated with pH as the primary factor, and also with other geochemical parameters such as Fe^{2+}, Fe^{3+} concentration and the redoxpotential.

Furthermore, pH as the most relevant geochemical parameter for determining the microbial community composition was confirmed in a recent experiment of natural pyrite oxidative dissolution simulating mineral weathering and acidification in sulfidic mine tailings (37). The microbial community at three stages of the weathering process was analyzed by pyrosequencing. At the early stage (pH > 5), the most dominant

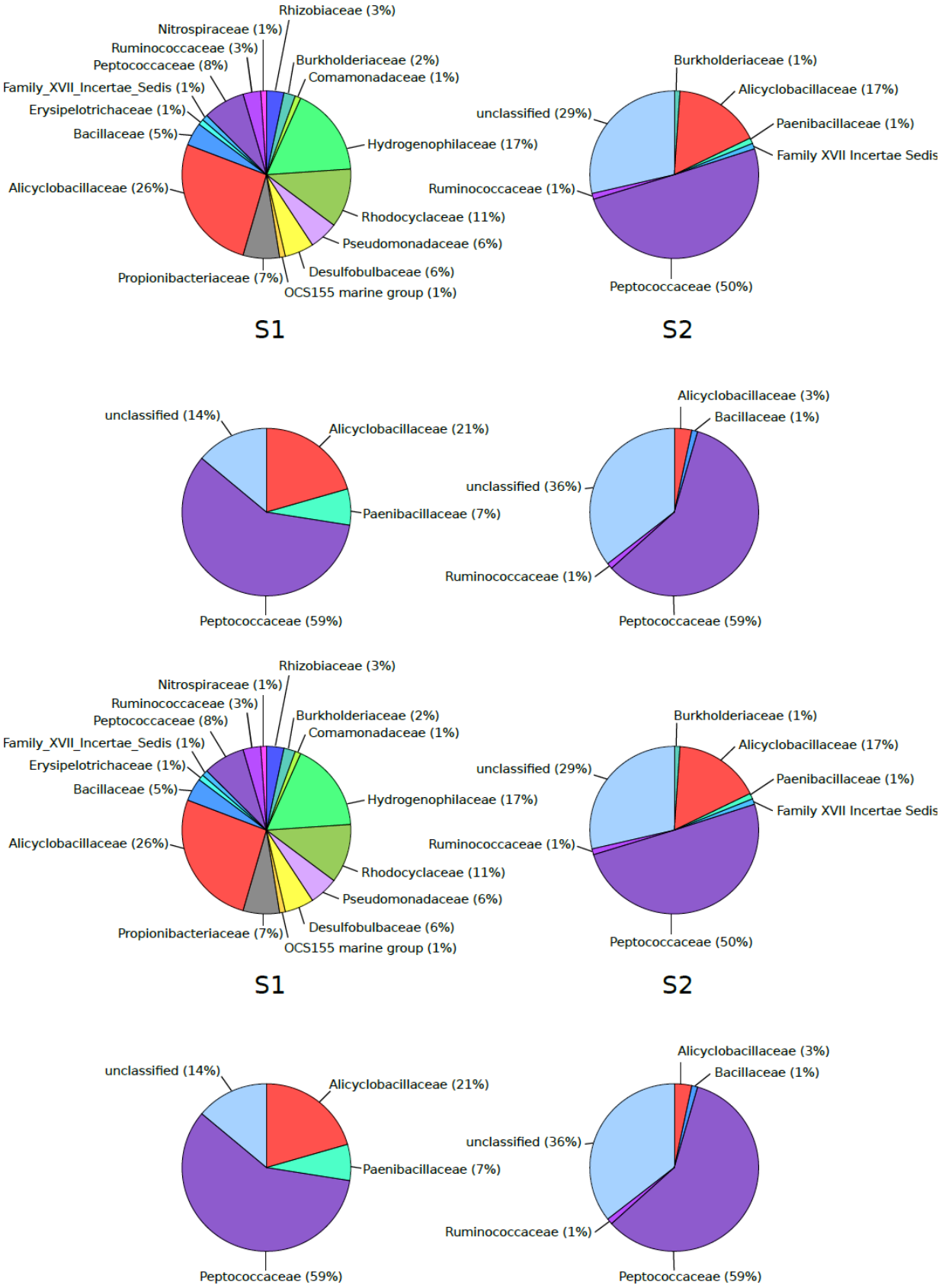


Fig. 1. Abundance of 16S rRNA gene clone phylotypes on the family level in the mine tailings from Selebi-Phikwe (Botswana, S1-S4), Kristineberg (Sweden, K1-K3) and Freiberg (Germany, F1).

genus was *Tumebacillus*, followed by *Thiobacillus*, *Brevundimonas*, *Dyella*, *Alicyclobacillus*, *Rubrobacter*, *Thiomonas*, *Bacillus*, *Sphingomonas*, and *Ferroplasma*. At the mid stage (5 > pH > 3), *Alicyclobacillus* became the most dominant genus. At the final stage below pH 3, a dramatic shift in the microbial community composition was detected. *Ferroplasma* became the most dominant genus, followed by *Leptospirillum*, *Sulfobacillus*, *Alicyclobacillus*, and *Acidithiobacillus*. The relative abundance of *Firmicutes*, *Proteobacteria*, and *Actinobacteria* significantly decreased below pH 3 and the community became dominated by *Euryarchaeota* and *Nitrospira*.

In conclusion our results show in line with the published literature that geochemical heterogeneous sulfidic mine tailings are characterized by complex bacterial communities at moderately acidic pH different to low pH communities which are dominated by acidophilic iron- and sulfur-oxidizing prokaryotes. However, other geochemical parameters besides pH also determine the composition of the microbial community composition in mine tailings.

Conflict of interest

We state that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.resmic.2014.08.007.

References

1. Kock D, Schippers A, Quantitative microbial community analysis of three different sulfidic mine tailing dumps generating acid mine drainage, Appl Environ Microbiol 74(16) (2008) 5211-5219.
2. Dold B, Fontboté L, Element cycling and secondary mineralogy in porphyry copper tailings as a function of climate, primary mineralogy, and mineral processing, Special issue: geochemical studies of mining and the environment, Geochem Explor 74 (1– 3) (2001) 3 – 55.

3. Blowes D W, Ptacek C J, Jurjovec J., Mill tailings: hydrogeology and geochemistry. In: Jambor J L, Blowes DW, Ritchie AIM (Eds.), Environmental aspects of mine wastes, Mineralogical Association of Canada 31 (2003) 95–116.
4. Schippers A, Breuker A, Blazejak A, Bosecker K, Kock D, Wright T L, The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria, Hydrometallurgy 104 (2010) 342-350.
5. Hedrich S, Schlömann M, Johnson D B, The iron-oxidizing proteobacteria, Microbiol J 157 (2011) 1551–1564.
6. Schippers A, Hedrich, Vasters J, Drobe M, Sand W, Willscher S, Biomining: metal recovery from ores with microorganisms. In: Geobiotechnology I - Metal-related Issues, A. Schippers, F. Glombitza, and W. Sand (eds.). Adv. Biochem. Eng. Biotechnol. 141 (2014), in press; online: http://link.springer.com/chapter/10.1007/10_2013_216/fulltext.html.
7. Baker B J, Banfield J F, Microbial communities in acid mine drainage, FEMS Microbiol Ecol 44 (2003) 139-152.
8. Gonzalez-Toril E, Llobet-Brossa E, Casamayor E O, Amann R, Amils R, Microbial ecology of an extreme acidic environment, the Tinto River, Appl Environ Microbiol 69 (2003) 4853-4865.
9. Johnson D B, Hallberg K B, The microbiology of acidic mine waters, Res Microbiol 154 (2003) 466-473.
10. Bruneel O, Duran R, Casiot C, Elbaz-Poulichet F, Personné J C, Diversity of microorganisms in Fe-As-rich acid mine drainage waters of Carnoulès, France, Appl Environ Microbiol 72 (2006) 551-556.
11. Johnson D B, Hallberg K B, Hedrich S, Uncovering a Microbial Enigma: Isolation and Characterization of the Streamer-Generating, Iron-Oxidizing, Acidophilic Bacterium "*Ferrovum myxofaciens*", Appl Environ Microbiol 80 (2014) 672-680.
12. Remonsellez F, Galleguillos F, Moreno-Paz M, Parro V, Acosta M, Demergasso C, Dynamic of active microorganisms inhabiting a bioleaching industrial heap of low-grade copper sulfide ore monitored by real-time PCR and oligonucleotide prokaryotic acidophile microarray, Microbial Biotechnol 2 (2009) 613–624.
13. Johnson D B, Geomicrobiology of extremely acidic subsurface environments, FEMS Microbiol Ecol 81 (2012) 2–12.
14. Ziegler S, Dolch K, Geiger K, Krause S, Asskamp M, Eusterhues K, Kriews M, Wilhelms-Dick D, Goettlicher J, Majzlan J, Gescher J, Oxygen-dependent niche formation of a pyrite-dependent acidophilic consortium built by archaea and bacteria. The ISME Journal 7 (2013) 1725-1737.
15. Southam G, Beveridge T J, Enumeration of thiobacilli within pH-neutral and acidic mine tailings and their role in the development of secondary mineral soil, Appl Environ Microbiol 58(1992) 1904-1912.
16. Fortin D, Davis B, Beveridge T J, Role of *Thiobacillus* and sulfate-reducing bacteria in iron biocycling in oxic and acidic mine tailings, Microbiol Ecol 21 (1996) 11-24.

17. Wielinga B, Juliette K L, Moore J N, Seastone O F, Cannon J E, Microbiological and geochemical characterization of fluvial deposited sulfidic mine tailings, Appl Environ Microbiol 65 (1999) 1548-1555.
18. Schippers A , Jozsa P G , Sand W , Kovacs Z M , Jelea M, Microbiological pyrite oxidation in a mine tailings heap and its relevance to the death of vegetation, Geomicrobiol J 17(2000) 151-162.
19. Graupner T, Kassahun A, Rammlmair D, Meima J, Kock D, Furche M, Fiege A, Schippers A, Melcher F, Formation of sequences of cemented layers and hardpans within sulfide-bearing mine tailings (mine district Freiberg, Germany), Appl Geochem 22 (2007) 2486–2508.
20. Tan G L, Shu W S , Hallberg K B , Li F , Lan C Y , Zhou W H , Huang L N, Culturable and molecular phylogenetic diversity of microorganisms in an open-dumped, extremely acidic Pb/Zn mine tailings, Extremophiles 12 (2008) 657–664.
21. Kock D, Schippers A, Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage, Hydrometallurgy 83 (2006) 167-175.
22. Diaby N, Dold B, Pfeifer H R, Holliger C, Johnson D B, Hallberg K B, Microbial communities in a porphyry copper tailings impoundment and their impact on the geochemical dynamics of the mine waste, Environ Microbiol 9 (2007) 298-307.
23. Zhang H B, Shi W, Yang M X, Sha T, Zhao Z W, Bacterial diversity at different depths in lead-zinc mine tailings as revealed by 16S rRNA gene libraries, J Microbiol 45 (2007) 479–484.
24. Mendez M O, Neilson J W, Maier R M, Characterization of a bacterial community in an abandoned semiarid lead-zinc mine tailings site, Appl Environ Microbiol 74 (2008) 3899-3907.
25. Winch S, Mills H J, Kostka J E, Fortin D, Lean D R S, Identification of sulfate-reducing bacteria in methylmercury- contaminated mine tailings by analysis of SSU rRNA genes. FEMS Microbiol Ecol 68 (2009) 94-107.
26. Huang L N, Zhou W H, Hallberg K B, Wan C Y, Li J, Shu W-S, Spatial and temporal analysis of the microbial community in the tailings of a Pb-Zn mine generating acidic drainage, Appl Environ Microbiol 77 (2011) 5540–5544.
27. Chen L X, Li J T, Chen Y T, Huang L N, Hua Z S, Hu M, Shu W S, Shifts in microbial community composition and function in the acidification of a lead/zinc mine tailings, Environ Microbiol 15 (2013) 2431–2444.
28. Liu J, Hua ZS, Chen ZN, Kuang JL, Li SJ, Shu WS, et al., Correlating microbial diversity patterns with geochemistry in an extreme and heterogeneous mine tailings environment, Appl Environ Microbiol 80 (2014) 3677-86.
29. Korehi H, Blöthe M, Sitnikova M A, Dold B, Schippers A, Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile, Environ Sci Technol 47 (2013) 2189-2196.
30. Schippers A, Kock D, Schwartz M O, Böttcher M E, Vogel H, Hagger M, Geomicrobiological and geochemical investigation of a pyrrhotite containing mine waste tailings dam near Selebi-Phikwe in Botswana, J Geochem Explor 92 (2007) 151-158.

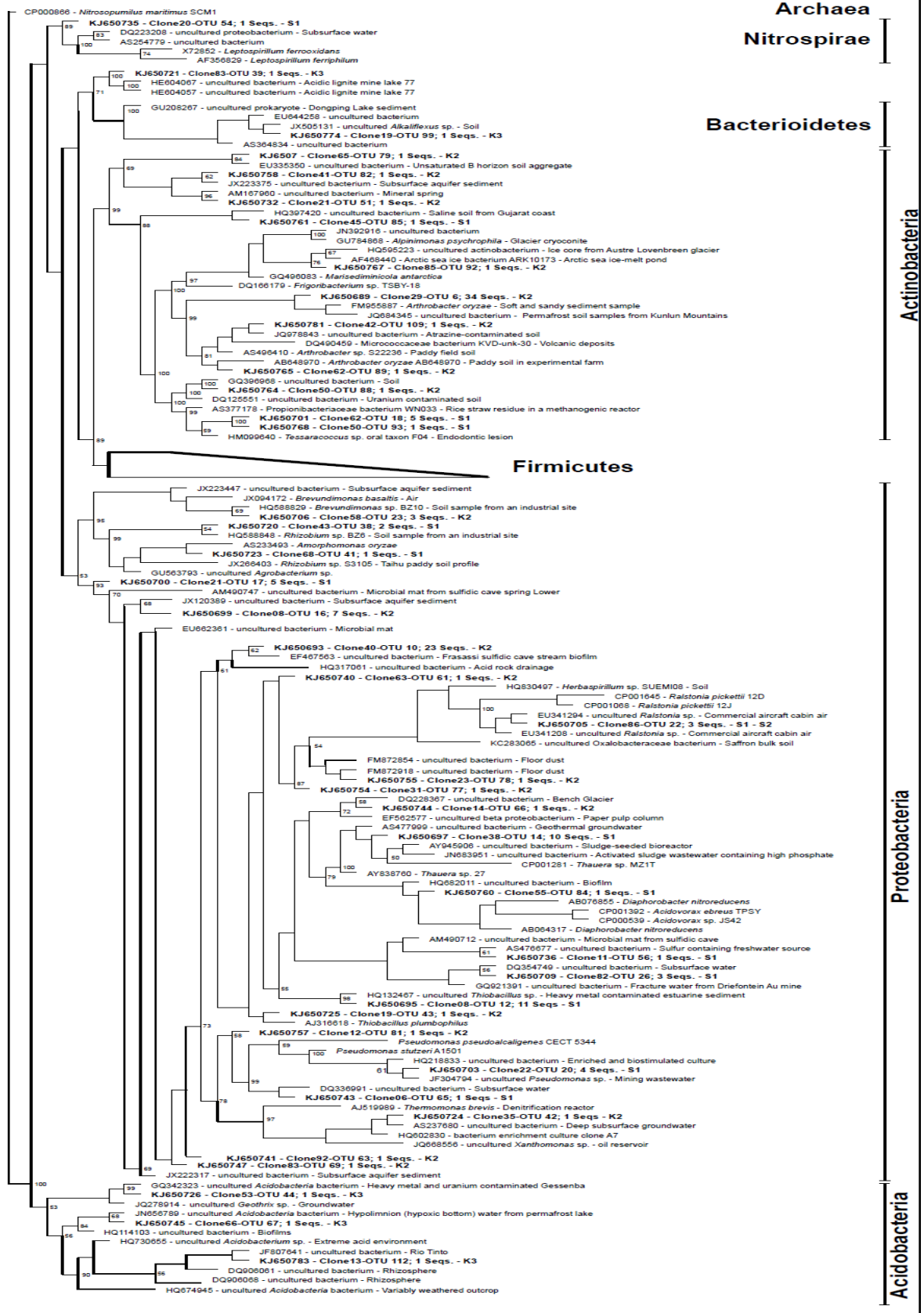
31. Herbert Jr, R B, Schippers A, Iron isotope fractionation by biogeochemical processes in mine tailings, Environ Sci Technol 42 (2008) 1117–1122.
32. Muyzer G, Teske A, Wirsen C O, Jannasch H W, Phylogenetic relationships of Thiomicrospira species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments, Arch Microbiol 164 (1995) 165-172.
33. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF, Introducing mothur: open-source, platform-independent community-supported software for describing and comparing microbial communities, Appl Environ Microbiol 75 (2009) 7537-7541.
34. Pruesse E, Peplies J, Glöckner FO, SINA: accurate high throughput multiple sequence alignment of ribosomal RNA genes, Bioinformatics 28 (2012) 1823-1829.
35. Stamatakis A, Aberer AJ, Goll C, Smith SA, Berger SA, Izquierdo-Carrasco F, RAxML-Light: A Tool for computing TeraByte Phylogenies, Bioinformatics 28 (2012) 2064-2066.
36. Miller M A, Pfeiffer W, Schwartz T, “Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, L A (2010) 1- 8.
37. Chen Y, Li J, Chen L, Hua J, Huang L, Liu J, et al. Biogeochemical processes governing natural pyrite oxidation and release of acid metalliferous drainage, Environ Sci Technol 48 (2014) 5537-45.
38. Pichtel JR, Dick WA. Sulfur, iron and solid phase transformations during biological oxidation of pyritic mine spoil. Soil Biol Biochem 23 (1991) 101-7.
39. Kaksonen A H, Spring S, Schumann P, Kroppenstedt R M, Puhakka J A, *Desulfurispora thermophilic* gen. nov., sp. nov., a thermophilic, spore-forming sulfate-reducer isolated from a sulfidogenic fluidized-bed reactor, Int J Syst Evol Microbiol 57 (2007) 1089-1094.
40. Fortin D, Praharaj T, Role of microbial activity in Fe and S cycling in sub-oxic to anoxic sulfide-rich mine tailings: a mini-review, J Nucl Radiochem Sci 6 (2005) 39-42.
41. Abicht H K, Mancini S, Karnachuk O V, Solioz M, Genome sequence of *Desulfosporosinus* sp. OT, an acidophilic sulfate-reducing bacterium from copper mining waste in Norilsk, northern Siberia, J Bacteriol 193(21) (2011) 6104-6105.
42. Pester M, Cheng J F, Tapia R, Wei C L, Markowitz V, Klenk H P, Brambilla E, Goodwin L, Land M L, Davenport K W, Szeto E, Spring S, Alazard D, Pitluck S, Hauser L, Ratte T, Peters L, Daligault H, Mikhailova N, Loy A, Weinmaier T, Ovchinnikova G, Kyrpides N C, Chain P S G, Pati A, Han J, Teshima H, Ivanova N N, Chen A, Wagner M, Lucas S,Detter J C, Pagani I, Lapidus A, Han C S, Huntmann M, Mavromatis K, Woyke T, Ollivier B, Complete Genome Sequences of *Desulfosporosinus orientis* DSM765^{T}, *Desulfosporosinus youngiae* DSM17734^{T}, *Desulfosporosinus meridiei* DSM13257^{T}, and *Desulfosporosinus acidiphilus* DSM22704^{T} , J Bacteriol 194 (2012) 6300-6301.
43. Navarro-Noyaa Y E, Hernández-Mendozaa E, Morales-Jiméneza J, Jan-Robleroa J, Martínez-Romerob E, Hernández-Rodrígueza C, Isolation and characterization of nitrogen fixing

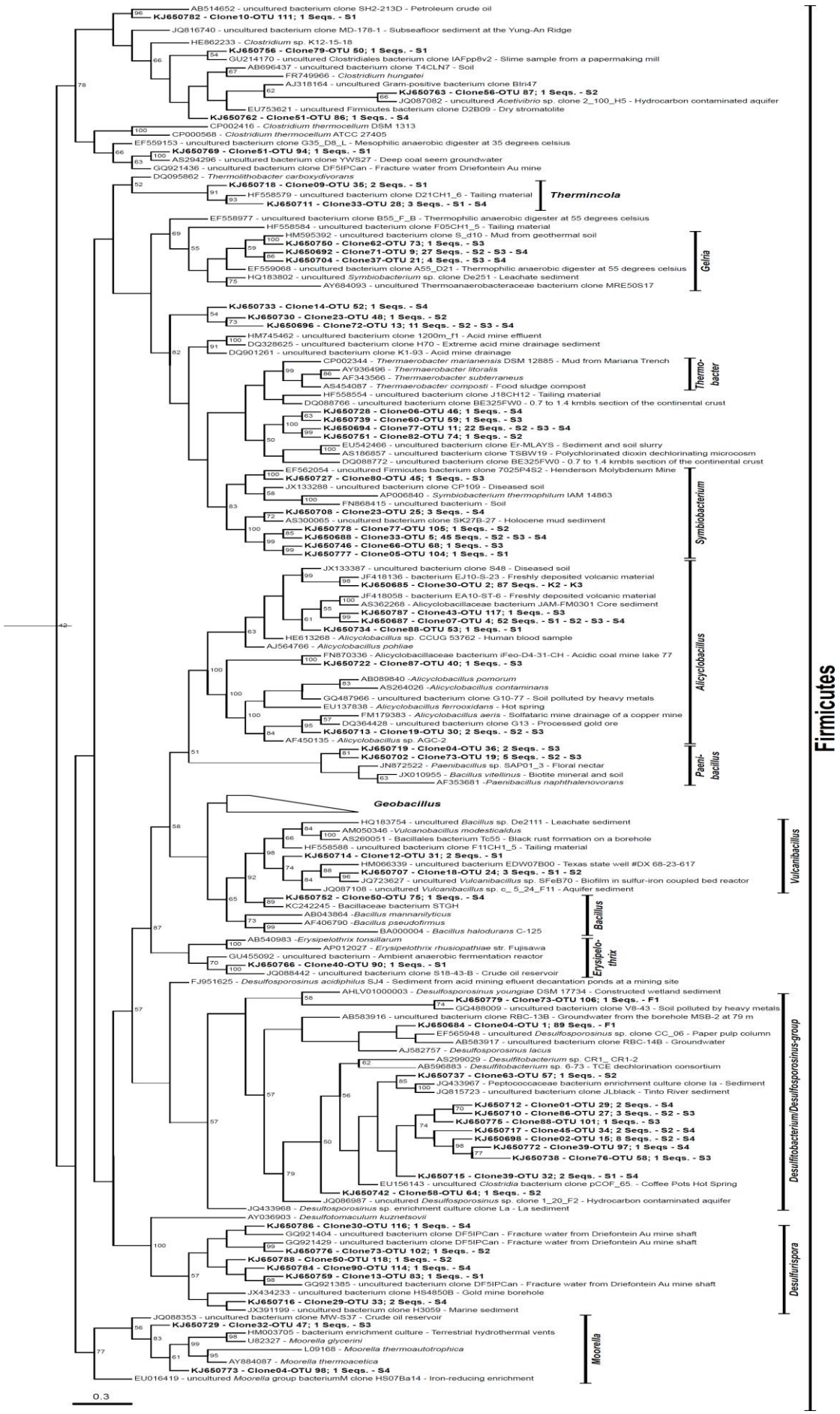
heterotrophic bacteria from the rhizosphere of pioneer plants growing on mine tailings, Appl Soil Ecol 62 (2012) 52–60.

4.4. Supplementary Material Figures

Figure S1 Phylogenetic tree (without the phylum Firmicutes) of representative 16S rRNA gene sequences retrieved from three different tailing dumps. One representative sequence per OTU is shown (in bold). Information of the relative abundances for each OTU and the appearance in which sample is given in the code preceeding the sequence names. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. Bootstrap values below 50% are not shown. The outgroup was *Nitrosopumilus maritimus* SCM1 (CP000866).

Figure S2 Phylogenetic tree of the of representative 16S rRNA gene sequences assigned only to the phylum Firmicutes retrieved from three different tailing dumps. One representative sequence per OTU is shown (in bold). Information of the relative abundances for each OTU and the appearance in which sample is given in the code preceeding the sequence names.


Archaea
Nitrospirae
Bacterioidetes
Actinobacteria
Firmicutes
Proteobacteria
Acidobacteria
CP000866 - Nitrosopumilus maritimus SCM1
KJ650735 - Clone20-OTU 54; 1 Seqs. - S1
DQ223208 - uncultured proteobacterium - Subsurface water
AS254779 - uncultured bacterium
X72852 - Leptospirillum ferrooxidans
AF356829 - Leptospirillum ferriphilum
KJ650721 - Clone83-OTU 39; 1 Seqs. - K3
HE604067 - uncultured bacterium - Acidic lignite mine lake 77
HE604057 - uncultured bacterium - Acidic lignite mine lake 77
GU208267 - uncultured prokaryote - Dongping Lake sediment
EU644258 - uncultured bacterium
JX505131 - uncultured Alkaliflexus sp. - Soil
KJ650774 - Clone19-OTU 99; 1 Seqs. - K3
AS364834 - uncultured bacterium
KJ6507 - Clone65-OTU 79; 1 Seqs. - K2
EU335350 - uncultured bacterium - Unsaturated B horizon soil aggregate
KJ650758 - Clone41-OTU 82; 1 Seqs. - K2
JX223375 - uncultured bacterium - Subsurface aquifer sediment
AM167960 - uncultured bacterium - Mineral spring
KJ650732 - Clone21-OTU 51; 1 Seqs. - K2
HQ397420 - uncultured bacterium - Saline soil from Gujarat coast
KJ650761 - Clone45-OTU 85; 1 Seqs. - S1
JN392916 - uncultured bacterium
GU784868 - Alpinimonas psychrophila - Glacier cryoconite
HQ595223 - uncultured actinobacterium - Ice core from Austre Lovenbreen glacier
AF468440 - Arctic sea ice bacterium ARK10173 - Arctic sea ice-melt pond
KJ650767 - Clone85-OTU 92; 1 Seqs. - K2
GQ496083 - Marisediminicola antarctica
DQ166179 - Frigoribacterium sp. TSBY-18
KJ650689 - Clone29-OTU 6; 34 Seqs. - K2
FM955887 - Arthrobacter oryzae - Soft and sandy sediment sample
JQ684345 - uncultured bacterium - Permafrost soil samples from Kunlun Mountains
KJ650781 - Clone42-OTU 109; 1 Seqs. - K2
JQ978843 - uncultured bacterium - Atrazine-contaminated soil
DQ490459 - Micrococcaceae bacterium KVD-unk-30 - Volcanic deposits
AS496410 - Arthrobacter sp. S22236 - Paddy field soil
AB648970 - Arthrobacter oryzae AB648970 - Paddy soil in experimental farm
KJ650765 - Clone62-OTU 89; 1 Seqs. - K2
GQ396968 - uncultured bacterium - Soil
KJ650764 - Clone50-OTU 88; 1 Seqs. - K2
DQ125551 - uncultured bacterium - Uranium contaminated soil
AS377178 - Propionibacteriaceae bacterium WN033 - Rice straw residue in a methanogenic reactor
KJ650701 - Clone62-OTU 18; 5 Seqs. - S1
KJ650768 - Clone50-OTU 93; 1 Seqs. - S1
HM099640 - Tessaracoccus sp. oral taxon F04 - Endodontic lesion
JX223447 - uncultured bacterium - Subsurface aquifer sediment
JX094172 - Brevundimonas basaltis - Air
HQ588829 - Brevundimonas sp. BZ10 - Soil sample from an industrial site
KJ650706 - Clone58-OTU 23; 3 Seqs. - K2
KJ650720 - Clone43-OTU 38; 2 Seqs. - S1
HQ588848 - Rhizobium sp. BZ6 - Soil sample from an industrial site
AS233493 - Amorphomonas oryzae
KJ650723 - Clone68-OTU 41; 1 Seqs. - S1
JX266403 - Rhizobium sp. S3105 - Taihu paddy soil profile
GU563793 - uncultured Agrobacterium sp.
KJ650700 - Clone21-OTU 17; 5 Seqs. - S1
AM490747 - uncultured bacterium - Microbial mat from sulfidic cave spring Lower
JX120389 - uncultured bacterium - Subsurface aquifer sediment
KJ650699 - Clone08-OTU 16; 7 Seqs. - K2
EU662361 - uncultured bacterium - Microbial mat
KJ650693 - Clone40-OTU 10; 23 Seqs. - K2
EF467563 - uncultured bacterium - Frasassi sulfidic cave stream biofilm
HQ317061 - uncultured bacterium - Acid rock drainage
KJ650740 - Clone63-OTU 61; 1 Seqs. - K2
HQ830497 - Herbaspirillum sp. SUEMI08 - Soil
CP001645 - Ralstonia pickettii 12D
CP001068 - Ralstonia pickettii 12J
EU341294 - uncultured Ralstonia sp. - Commercial aircraft cabin air
KJ650705 - Clone86-OTU 22; 3 Seqs. - S1 - S2
EU341208 - uncultured Ralstonia sp. - Commercial aircraft cabin air
KC283065 - uncultured Oxalobacteraceae bacterium - Saffron bulk soil
FM872854 - uncultured bacterium - Floor dust
FM872918 - uncultured bacterium - Floor dust
KJ650755 - Clone23-OTU 78; 1 Seqs. - K2
KJ650754 - Clone31-OTU 77; 1 Seqs. - K2
DQ228367 - uncultured bacterium - Bench Glacier
KJ650744 - Clone14-OTU 66; 1 Seqs. - K2
EF562577 - uncultured beta proteobacterium - Paper pulp column
AS477999 - uncultured bacterium - Geothermal groundwater
KJ650697 - Clone38-OTU 14; 10 Seqs. - S1
AY945906 - uncultured bacterium - Sludge-seeded bioreactor
JN683951 - uncultured bacterium - Activated sludge wastewater containing high phosphate
CP001281 - Thauera sp. MZ1T
AY838760 - Thauera sp. 27
HQ682011 - uncultured bacterium - Biofilm
KJ650760 - Clone55-OTU 84; 1 Seqs. - S1
AB076855 - Diaphorobacter nitroreducens
CP001392 - Acidovorax ebreus TPSY
CP000539 - Acidovorax sp. JS42
AB064317 - Diaphorobacter nitroreducens
AM490712 - uncultured bacterium - Microbial mat from sulfidic cave
AS476677 - uncultured bacterium - Sulfur containing freshwater source
KJ650736 - Clone11-OTU 56; 1 Seqs. - S1
DQ354749 - uncultured bacterium - Subsurface water
KJ650709 - Clone82-OTU 26; 3 Seqs. - S1
GQ921391 - uncultured bacterium - Fracture water from Driefontein Au mine
HQ132467 - uncultured Thiobacillus sp. - Heavy metal contaminated estuarine sediment
KJ650695 - Clone08-OTU 12; 11 Seqs - S1
KJ650725 - Clone19-OTU 43; 1 Seqs. - K2
AJ31618 - Thiobacillus plumbophilus
KJ650757 - Clone12-OTU 81; 1 Seqs - K2
Pseudomonas pseudoalcaligenes CECT 5344
Pseudomonas stutzeri A1501
HQ218833 - uncultured bacterium - Enriched and biostimulated culture
KJ650703 - Clone22-OTU 20; 4 Seqs. - S1
JF304794 - uncultured Pseudomonas sp. - Mining wastewater
DQ336991 - uncultured bacterium - Subsurface water
KJ650743 - Clone06-OTU 65; 1 Seqs - S1
AJ519989 - Thermomonas brevis - Denitrification reactor
KJ650724 - Clone35-OTU 42; 1 Seqs - K2
AS237680 - uncultured bacterium - Deep subsurface groundwater
HQ602830 - bacterium enrichment culture clone A7
JQ668556 - uncultured Xanthomonas sp. - oil reservoir
KJ650741 - Clone92-OTU 63; 1 Seqs. - K2
KJ650747 - Clone83-OTU 69; 1 Seqs. - K2
JX222317 - uncultured bacterium - Subsurface aquifer sediment
GQ342323 - uncultured Acidobacteria bacterium - Heavy metal and uranium contaminated Gessenba
KJ650726 - Clone53-OTU 44; 1 Seqs. - K3
JQ278914 - uncultured Geothrix sp. - Groundwater
JN656789 - uncultured Acidobacteria bacterium - Hypolimnion (hypoxic bottom) water from permafrost lake
KJ650745 - Clone66-OTU 67; 1 Seqs. - K3
HQ114103 - uncultured bacterium - Biofilms
HQ730655 - uncultured Acidobacterium sp. - Extreme acid environment
JF807641 - uncultured bacterium - Rio Tinto
KJ650783 - Clone13-OTU 112; 1 Seqs. - K3
DQ906061 - uncultured bacterium - Rhizosphere
DQ906068 - uncultured bacterium - Rhizosphere
HQ674945 - uncultured Acidobacteria bacterium - Variably weathered outcrop
0.20


AB514652 - uncultured bacterium clone SH2-213D - Petroleum crude oil
KJ650782 - Clone10-OTU 111; 1 Seqs. - S1
JQ816740 - uncultured bacterium clone MD-178-1 - Subseafloor sediment at the Yung-An Ridge
HE862233 - Clostridium sp. K12-15-18
KJ650756 - Clone79-OTU 50; 1 Seqs. - S1
GU214170 - uncultured Clostridiales bacterium clone IAFpp8v2 - Slime sample from a papermaking mill
AB696437 - uncultured bacterium clone T4CLN7 - Soil
FR749966 - Clostridium hungatei
AJ318164 - uncultured Gram-positive bacterium clone Blri47
KJ650763 - Clone56-OTU 87; 1 Seqs. - S2
JQ087082 - uncultured Acetivibrio sp. clone 2_100_H5 - Hydrocarbon contaminated aquifer
EU753621 - uncultured Firmicutes bacterium clone D2B09 - Dry stromatolite
KJ650762 - Clone51-OTU 86; 1 Seqs. - S4
CP002416 - Clostridium thermocellum DSM 1313
CP000568 - Clostridium thermocellum ATCC 27405
EF559153 - uncultured bacterium clone G35_D8_L - Mesophilic anaerobic digester at 35 degrees celsius
KJ650769 - Clone51-OTU 94; 1 Seqs. - S1
AS294296 - uncultured bacterium clone YWS27 - Deep coal seem groundwater
GQ921436 - uncultured bacterium clone DF5IPCan - Fracture water from Driefontein Au mine
DQ095862 - Thermolithobacter carboxydivorans
KJ650718 - Clone09-OTU 35; 2 Seqs. - S1
HF558579 - uncultured bacterium clone D21CH1_6 - Tailing material
KJ650711 - Clone33-OTU 28; 3 Seqs. - S1 - S4
Thermincola
EF558977 - uncultured bacterium clone B55_F_B - Thermophilic anaerobic digester at 55 degrees celsius
HF558584 - uncultured bacterium clone F05CH1_5 - Tailing material
HM595392 - uncultured bacterium clone S_d10 - Mud from geothermal soil
KJ650750 - Clone62-OTU 73; 1 Seqs. - S3
KJ650692 - Clone71-OTU 9; 27 Seqs. - S2 - S3 - S4
KJ650704 - Clone37-OTU 21; 4 Seqs. - S3 - S4
EF559068 - uncultured bacterium clone A55_D21 - Thermophilic anaerobic digester at 55 degrees celsius
HQ183802 - uncultured Symbiobacterium sp. clone De251 - Leachate sediment
AY684093 - uncultured Thermoanaerobacteraceae bacterium clone MRE50S17
Geliria
KJ650733 - Clone14-OTU 52; 1 Seqs. - S4
KJ650730 - Clone23-OTU 48; 1 Seqs. - S2
KJ650696 - Clone72-OTU 13; 11 Seqs. - S2 - S3 - S4
HM745462 - uncultured bacterium clone 1200m_f1 - Acid mine effluent
DQ328625 - uncultured bacterium clone H70 - Extreme acid mine drainage sediment
DQ901261 - uncultured bacterium clone K1-93 - Acid mine drainage
CP002344 - Thermaerobacter marianensis DSM 12885 - Mud from Mariana Trench
AY936496 - Thermaerobacter litoralis
AF343566 - Thermaerobacter subterraneus
AS454087 - Thermaerobacter composti - Food sludge compost
HF558554 - uncultured bacterium clone J18CH12 - Tailing material
DQ088766 - uncultured bacterium clone BE325FW0 - 0.7 to 1.4 kmbls section of the continental crust
Thermo-bacter
KJ650728 - Clone06-OTU 46; 1 Seqs. - S4
KJ650739 - Clone60-OTU 59; 1 Seqs. - S3
KJ650694 - Clone77-OTU 11; 22 Seqs. - S2 - S3 - S4
KJ650751 - Clone82-OTU 74; 1 Seqs. - S2
EU542466 - uncultured bacterium clone Er-MLAYS - Sediment and soil slurry
AS186857 - uncultured bacterium clone TSBW19 - Polychlorinated dioxin dechlorinating microcosm
DQ088772 - uncultured bacterium clone BE325FW0 - 0.7 to 1.4 kmbls section of the continental crust
EF562054 - uncultured Firmicutes bacterium clone 7025P4S2 - Henderson Molybdenum Mine
KJ650727 - Clone80-OTU 45; 1 Seqs. - S3
JX133288 - uncultured bacterium clone CP109 - Diseased soil
AP006840 - Symbiobacterium thermophilum IAM 14863
FN868415 - uncultured bacterium - Soil
KJ650708 - Clone23-OTU 25; 3 Seqs. - S4
AS300065 - uncultured bacterium clone SK27B-27 - Holocene mud sediment
KJ650778 - Clone77-OTU 105; 1 Seqs. - S2
KJ650688 - Clone33-OTU 5; 45 Seqs. - S2 - S3 - S4
KJ650746 - Clone66-OTU 68; 1 Seqs. - S3
KJ650777 - Clone05-OTU 104; 1 Seqs. - S1
Symbiobacterium
JX133387 - uncultured bacterium clone S48 - Diseased soil
JF418136 - bacterium EJ10-S-23 - Freshly deposited volcanic material
KJ650685 - Clone30-OTU 2; 87 Seqs. - K2 - K3
JF418058 - bacterium EA10-ST-6 - Freshly deposited volcanic material
AS362268 - Alicyclobacillaceae bacterium JAM-FM0301 Core sediment
KJ650787 - Clone43-OTU 117; 1 Seqs. - S3
KJ650687 - Clone07-OTU 4; 52 Seqs. - S1 - S2 - S3 - S4
KJ650734 - Clone88-OTU 53; 1 Seqs. - S1
HE613268 - Alicyclobacillus sp. CCUG 53762 - Human blood sample
AJ564766 - Alicyclobacillus pohliae
FN870336 - Alicyclobacillaceae bacterium iFeo-D4-31-CH - Acidic coal mine lake 77
KJ650722 - Clone87-OTU 40; 1 Seqs. - S3
AB089840 -Alicyclobacillus pomorum
AS264026 -Alicyclobacillus contaminans
GQ487966 - uncultured bacterium clone G10-77 - Soil polluted by heavy metals
EU137838 - Alicyclobacillus ferrooxidans - Hot spring
FM179383 - Alicyclobacillus aeris - Solfataric mine drainage of a copper mine
DQ364428 - uncultured bacterium clone G13 - Processed gold ore
KJ650713 - Clone19-OTU 30; 2 Seqs. - S2 - S3
AF450135 - Alicyclobacillus sp. AGC-2
Alicyclobacillus
KJ650719 - Clone04-OTU 36; 2 Seqs. - S3
KJ650702 - Clone73-OTU 19; 5 Seqs. - S2 - S3
JN872522 - Paenibacillus sp. SAP01_3 - Floral nectar
JX010955 - Bacillus vitellinus - Biotite mineral and soil
AF353681 -Paenibacillus naphthalenovorans
Paeni-bacillus
Geobacillus
HQ183754 - uncultured Bacillus sp. De2111 - Leachate sediment
AM050346 -Vulcanobacillus modesticaldus
AS260051 - Bacillales bacterium Tc55 - Black rust formation on a borehole
HF558588 - uncultured bacterium clone F11CH1_5 - Tailing material
KJ650714 - Clone12-OTU 31; 2 Seqs. - S1
HM066339 - uncultured bacterium EDW07B00 - Texas state well #DX 68-23-617
KJ650707 - Clone18-OTU 24; 3 Seqs. - S1 - S2
JQ723627 - uncultured Vulcanibacillus sp. SFeB70 - Biofilm in sulfur-iron coupled bed reactor
JQ087108 - uncultured Vulcanibacillus sp. c_ 5_24_F11 - Aquifer sediment
Vulcanibacillus
KJ650752 - Clone50-OTU 75; 1 Seqs. - S4
KC242245 - Bacillaceae bacterium STGH
AB043864 -Bacillus mannanilyticus
AF406790 -Bacillus pseudofirmus
BA000004 - Bacillus halodurans C-125
Bacillus
AB540983 -Erysipelothrix tonsillarum
AP012027 - Erysipelothrix rhusiopathiae str. Fujisawa
GU455092 - uncultured bacterium - Ambient anaerobic fermentation reactor
KJ650766 - Clone40-OTU 90; 1 Seqs. - S1
JQ088442 - uncultured bacterium clone S18-43-B - Crude oil reservoir
Erysipelo-thrix
FJ951625 - Desulfosporosinus acidiphilus SJ4 - Sediment from acid mining effluent decantation ponds at a mining site
AHLV01000003 - Desulfosporosinus youngiae DSM 17734 - Constructed wetland sediment
KJ650779 - Clone73-OTU 106; 1 Seqs. - F1
GQ488009 - uncultured bacterium clone V8-43 - Soil polluted by heavy metals
AB583916 - uncultured bacterium clone RBC-13B - Groundwater from the borehole MSB-2 at 79 m
KJ650684 - Clone04-OTU 1; 89 Seqs. - F1
EF565948 - uncultured Desulfosporosinus sp. clone CC_06 - Paper pulp column
AB583917 - uncultured bacterium clone RBC-14B - Groundwater
AJ582757 - Desulfosporosinus lacus
AS299029 - Desulfitobacterium sp. CR1_ CR1-2
AB596883 - Desulfitobacterium sp. 6-73 - TCE dechlorination consortium
KJ650737 - Clone63-OTU 57; 1 Seqs. - S2
JQ433967 - Peptococcaceae bacterium enrichment culture clone la - Sediment
JQ815723 - uncultured bacterium clone JLblack - Tinto River sediment
KJ650712 - Clone01-OTU 29; 2 Seqs. - S4
KJ650710 - Clone86-OTU 27; 3 Seqs. - S2 - S3
KJ650775 - Clone88-OTU 101; 1 Seqs. - S3
KJ650717 - Clone45-OTU 34; 2 Seqs. - S2 - S4
KJ650698 - Clone02-OTU 15; 8 Seqs. - S2 - S4
KJ650772 - Clone39-OTU 97; 1 Seqs - S4
KJ650738 - Clone76-OTU 58; 1 Seqs. - S3
KJ650715 - Clone39-OTU 32; 2 Seqs. - S1 - S4
EU156143 - uncultured Clostridia bacterium clone pCOF_65. - Coffee Pots Hot Spring
KJ650742 - Clone58-OTU 64; 1 Seqs. - S2
JQ086987 - uncultured Desulfosporosinus sp. clone 1_20_F2 - Hydrocarbon contaminated aquifer
JQ433968 - Desulfosporosinus sp. enrichment culture clone La - La sediment
Desulfitobacterium/Desulfosporosinus-group
AY036903 - Desulfotomaculum kuznetsovii
KJ650786 - Clone30-OTU 116; 1 Seqs. - S4
GQ921404 - uncultured bacterium clone DF5IPCan - Fracture water from Driefontein Au mine shaft
GQ921429 - uncultured bacterium clone DF5IPCan - Fracture water from Driefontein Au mine shaft
KJ650776 - Clone73-OTU 102; 1 Seqs. - S2
KJ650788 - Clone50-OTU 118; 1 Seqs. - S2
KJ650784 - Clone90-OTU 114; 1 Seqs. - S4
KJ650759 - Clone13-OTU 83; 1 Seqs. - S1
GQ921385 - uncultured bacterium clone DF5IPCan - Fracture water from Driefontein Au mine shaft
JX434233 - uncultured bacterium clone HS4850B - Gold mine borehole
KJ650716 - Clone29-OTU 33; 2 Seqs. - S4
JX391199 - uncultured bacterium clone H3059 - Marine sediment
Desulfurispora
JQ088353 - uncultured bacterium clone MW-S37 - Crude oil reservoir
KJ650729 - Clone32-OTU 47; 1 Seqs. - S3
HM003705 - bacterium enrichment culture - Terrestrial hydrothermal vents
U82327 - Moorella glycerini
L09168 - Moorella thermoautotrophica
AY884087 - Moorella thermoacetica
KJ650773 - Clone04-OTU 98; 1 Seqs. - S4
EU016419 - uncultured Moorella group bacteriumM clone HS07Ba14 - Iron-reducing enrichment
Moorella
Firmicutes
0.3

Chapter 5

Bioleaching of a marine hydrothermal sulfide ore with mesophiles, moderate thermophiles and thermophiles **(Advanced Materials Research 825 (2013) 329-332)**

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Abstract

Marine hydrothermal polymetallic sulfide ores contain high amounts of valuable metals such as Cu, Pb, Zn, Au, Ag, as well as In, Ge, Bi, and Se. Samples from a site in the Indian Ocean were taken during a BGR ship cruise, crushed and sieved for bioleaching experiments to reveal the extraction of the various metals. Chalcopyrite was the main mineral, the total copper content was 38.5 %wt. Comparative bioleaching with mesophilic, moderate thermophilic and thermophilic acidophilic iron- and sulfur-oxidizing bacteria and archaea was investigated. Batch culture experiments were conducted at 2% (w/v) pulp density in shake flasks in the presence of *Acidithiobacillus ferrooxidans, Acidiphilium* sp. and *Acidithiobacillus thiooxidans* as mesophiles (30°C), a mixed culture of moderate thermopilic iron- and sulfur oxidizing bacteria (50°C) and the thermophile *Acidianus brierleyi* (70°C)**.** The results after four weeks showed most effective dissolution of copper in the presence of *A. brierleyi* (up to 4.3 g/l), compared with moderate thermophiles and mesophiles (3.3 g/l and 2.5 g/l, respectively). Furthermore, the bioleaching performance was approved with dissolved iron concentrations. Conclusively, an increase in temperature from 30°C to 70°C had a major impact on bioleaching efficiency. Copper and iron extraction efficiency occurred in the order thermophiles, moderate thermophiles, and mesophiles.

5.1. Introduction

Marine hydrothermal activity is a major mechanism for concentration of metals, which accumulate in the form of polymetallic sulfides very rich in Cu, Pb, Zn, Au, Ag, etc. Metal sulfides can be oxidized during autotrophic growth of mesophilic, moderate thermophilic and thermophilic bacteria and archaea (1). Chalcopyrite represents the largest copper reservoir in the world, but as a refractory primary copper sulfide its

dissolution via bioleaching is challenging (2-6). Since several studies with mesophilic microorganisms have shown very low copper leaching rates, many researchers have examined the potential of using thermophilic microorganism which play an important role in biooxidations of minerals, especially in the case of chalcopyrite (3, 6,7). This paper describes bioleaching of chalcopyrite as main mineral in a marine hydrothermal polymetallic sulfide ore. The mining of such ores is under discussion and bioleaching as a potential processing step is tested. Considering the literature about chalcopyrite bioleaching, experiments at different temperatures using mesophilic, moderate thermophilic and thermophilic acidophilic iron- and sulfur-oxidizing bacteria and archaea were carried out.

5.2. Materials and methods

Marine hydrothermal polymetallic sulfide samples from the Indian Ocean were collected during a BGR ship cruise, and crushed and sieved (50-200 µm) for bioleaching experiments. Quantitative chemical analysis showed that the massive copper ores contained about 35 %wt Fe and 38.5 %wt Cu. The main mineral was chalcopyrite. Minor minerals included pyrite, sphalerite and marcasite.

Comparative bioleaching experiments were performed using the three following inoculums; a mixed mesophilic culture of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Acidiphilium* sp. from BGR culture collection, a mixed moderate thermophilic culture of unknown iron- and sulfur-oxidizing bacteria from South Africa as well as *Acidianus brierleyi* from the German Collection of Microorganism and Cell Cultures (DSMZ) as thermophilic archaea. Mesophilic microorganisms were cultivated in Leathen medium, moderate thermophiles and thermophiles were grown in 9K and DSM 150 media, respectively.

The bioleaching experiments were carried out in 500 ml Erlenmeyer flasks on rotary shakers (150 rpm) in three parallels; each flask contained 200 ml of the proper medium as well as 2 % w/v of solids. After the pH was adjusted, experiments were started with 10 % (v/v) inoculum of the mesophiles, moderate thermophiles or thermophiles. The incubation temperatures were 30°C, 50°C and 70°C, respectively. The loss of water due to evaporation was compensated by adding sterilised distilled water after weighting the flasks regularly. The stock and pre-inoculum cultures were adapted and maintained in the same media under the similar conditions that are described above. In addition to bioleaching assays, chemical controls treated with 2% thymol-ethanol were run. The initial pH of the cultures was adjusted for the respective cultures and kept below pH 3.5 during the experiments by adding 3 M H_2SO_4 when necessary. Samples were taken on a regular basis and analysed for soluble copper and iron concentration, pH, redox potential (vs. Ag/AgCl), phase contrast microscopic cell counting, and the number of viable cells via most-probable-number (MPN) cultivation in the respective media and temperatures.

5.3. Results

Copper bioleaching from a chalcopyrite-rich marine hydrothermal polymetallic sulfide ore at different temperatures using mesophilic (30°C), moderately thermophilic (50°C) and thermophilic (70°C) microorganisms was investigated. The results of Cu^{+2} extractions at different temperatures are shown in Fig. 1. The results showed a low dissolution of copper with the mesophiles and thermophiles within the first week, while the moderate thermophiles showed increasing copper extraction within the first fifteen days and constant values towards the end of the experiment. Cu^{+2} extraction proceeded at similar rates with mesophiles at 30°C and thermophiles at 70°C within

the first week. After four weeks most effective dissolution of copper occurred in presence of *A. brierleyi* (4.3 g/l), compared with the moderate thermophiles and mesophiles (3.3 g/l and 2.5 g/l, respectively). A copper extraction of 56 %, 42 % and 32 % was measured for the thermophiles, moderate thermophiles and mesophiles, respectively. The bioleaching experiment for the mesophiles was run longer for six weeks whereby 3 g/L copper were leached (data not shown). Copper leaching under abiotic conditions was not significant.

The bioleaching performance was approved with dissolved iron concentrations which were highest for the thermophiles, intermediate for the moderate thermophiles and lowest for the mesophiles (data not shown). Iron plays a key role in the bioleaching processes and its oxidation state is the main factor to determine redox potential. The redox potential values showed an increase in all bioleaching cultures but in case of the thermophile the increase was significantly lower (to maximal 400 mV vs. Ag/AgCl) than for the other cultures (Fig. 2). The increase of the redox potential during bioleaching process corresponded overall with the increase of the concentration of Cu^{+2} in solution (Fig. 1).

The initially adjusted pH changed during mineral oxidation as a consequence of biotic and abiotic reactions (Fig. 3). The planktonic total cell counts for the cultures during the bioleaching experiments shown in Fig. 3 did not correspond with the copper bioleaching activity. Highest counts were observed for the mesophiles, followed by the thermophiles, while hardly any increase of cell counts was detected for the moderate thermophiles. One has to consider that only planktonic cells were counted, cells attached to the mineral, likely more important for copper dissolution, were not counted. However, for all cultures, cultivable living iron- and sulfur-oxidizing cells were detected throughout the experiments via the MPN technique (data not shown).

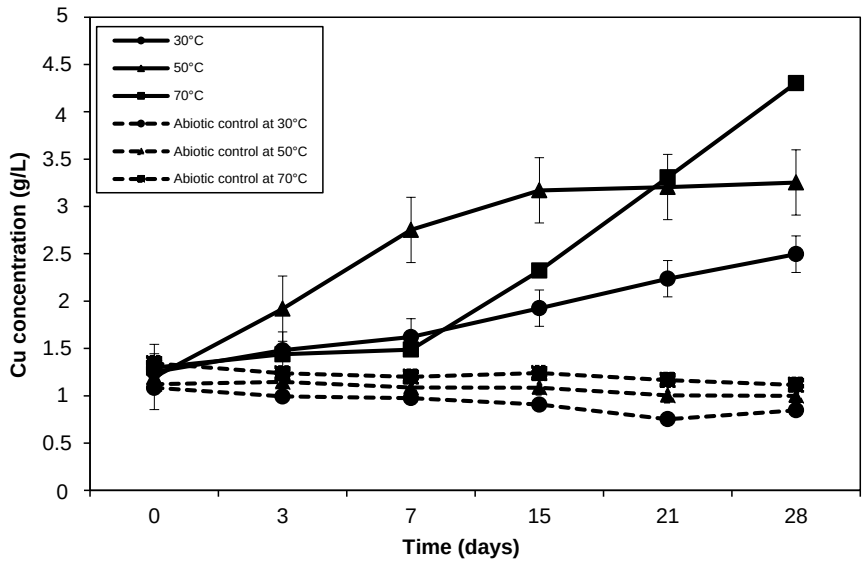

Fig. 1. Cu dissolution during bioleaching of 2 % (w/v) chalcopyrite-rich marine hydrothermal polymetallic sulfide ore by cultures of mesophilic (30°C), moderate thermophilic (50°C) and thermophilic (70°C) bacteria and archaea.

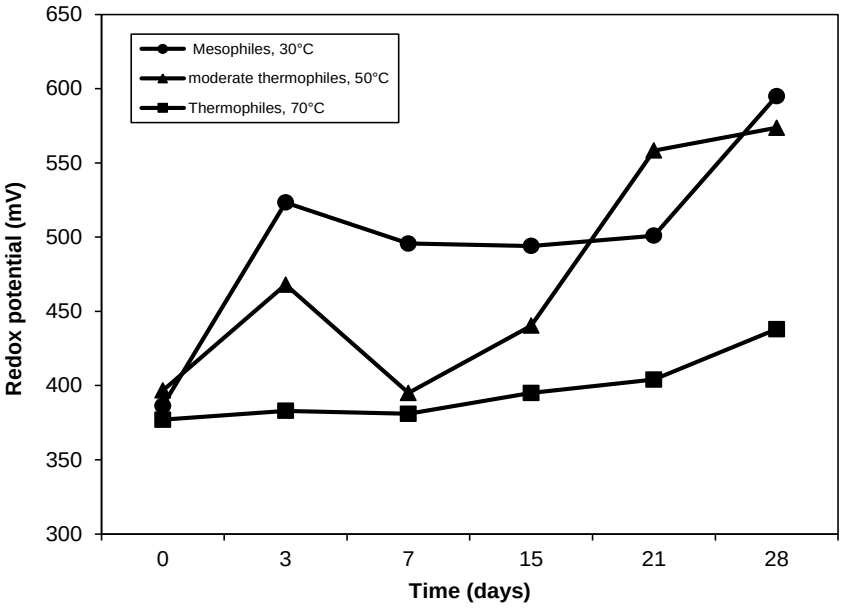

Fig. 2. Redox potential during bioleaching of 2% (w/v) chalcopyrite-rich marine hydrothermal polymetallic sulfide ore with of mesophiles (30°C), moderate thermophiles (50°C) and thermophiles (70°C).

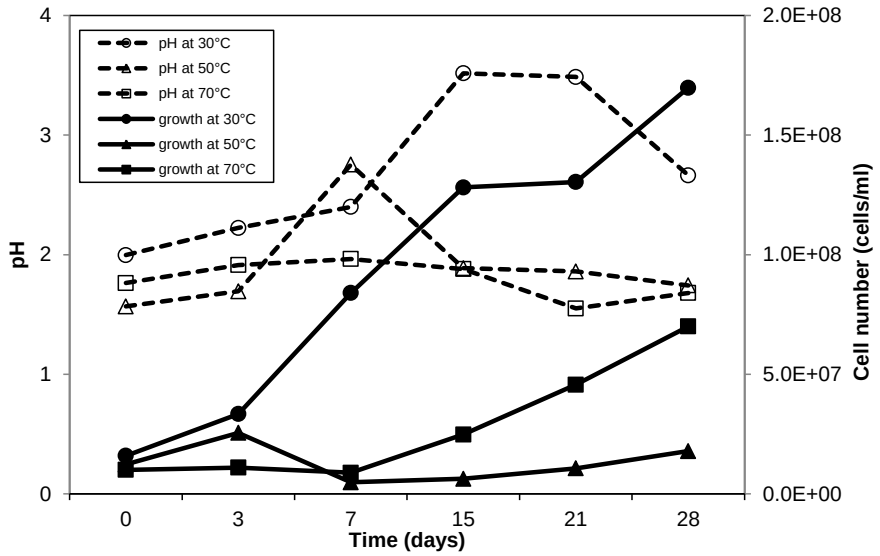

Fig. 3. Total cell counts and pH in bioleaching experiments with mesophiles (30°C), moderate thermophiles (50°C) and thermophiles (70°C).

5.4. Discussion

Numerous acidophilic bacteria and archaea can oxidize sulfidic minerals due to their abilities to oxidise reduced iron and/or sulfur compounds. However, the rate of sulfide mineral dissolution depends on physiological characteristics of the microorganisms. Accordingly, metal sulfide oxidation process was investigated by mesophilic (30°C), moderate thermophilic (50°C) and thermophilic (70°C) cultures, in which a chalcopyrite-rich marine hydrothermal polymetallic sulfide ore was oxidized as the sole substrate. Copper dissolution was maximal with the thermophile *Acidianus brierleyi* at 70°C according to published results (1,6). However, mesophiles exhibited a shorter lag phase. Rawlings (2005) (7) discussed that the growth of moderate thermophiles can be limited by CO_2 uptake due to a reduced CO_2 solubility at 50°C. Konishi et al. (6) discussed attachment of cells on the surface of metal sulfides explaining a decline of planktonic cells in the cultures. Compared to mesophilic and moderate thermophilic bioleaching microorganisms, the thermophiles adjusted a lower redox potential.

Sandström et al. (8) showed that chalcopyrite leaches more readily at lower redox potential.

5.5. Summary

An increase in temperature from 30℃ to 70℃ had a major impact on bioleaching efficiency in bioleaching shake flask experiments of a marine hydrothermal chalcopyrite ore. Copper and iron extraction efficiency occurred in the order thermophiles, moderate thermophiles, and mesophiles.

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References

1. D.A. Clarck, P.R. Norris, Oxidation of mineral sulphides by thermophilic microorganisms, Min. Eng. 11 (1996) 1119-1125.
2. P. Gonzalez-Contreras, J. Weijma, C.J.N. Buisman, Kinetics of ferrous iron oxidation by batch and continuous cultures of thermoacidophilic *Archaea* at extremely low pH of 1.1-1.3, Appl. Microbiol. Biotechnol. 93 (2012) 1295-1303.
3. A. Rubio, F.J. Garcia Frutos, Bioleaching capacity of an extremely thermophilic culture for chalcopyrite materials. Min. Eng. 15 (2002) 689-694.
4. J. Petersen, D.G. Dixon, Competitive bioleaching of pyrite and chalcopyrite, Hydrometallurgy 83 (2006) 40-49.
5. M. Gericke, A. Pinches, J.V. van Rooyen, Bioleaching of chalcopyrite concentrate using an extremely thermophilic culture, Int.J. Miner. Process. 62 (2001) 243-255.
6. Y. Konishi, M. Tokushige, S. Asai, T. Suzuki, Copper recovery from chalcopyrite concentrate by acidophilic thermophile *Acidianus brierleyi* in batch and continuous-flow stirred tank reactors, Hydrometallurgy 59 (2001) 271-282.
7. D.E. Rawlings, Characteristics and adaptability of iron- and sulfur–oxidizing microorganisms used for the recovery of metals from minerals and their concentrates, Microbial cell factories 4 (2005) 13.
8. A. Sandström, A. Shchukarev, J. Paul, XPS characterisation of chalcopyrite chemically and bioleached at high and low redox potential. Min. Eng. 18 (2005) 505-515.

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Erklärung zur Dissertation

Gemäß §6(1) der Promotionordnung der Naturwissenschaftlichen Fakultät der Gottfried Wilhelm Leibniz Universität Hannover Für die Promotion zum Dr. rer. nat.

Hierdurch erkläre ich, daß ich meine Dissertation mit dem Titel

„Microbial diversity in mine tailings and the role of metal sulfide oxidizers in biomining processes“

selbständig verfaßt und die benutzten Hilfsmittel und Quellen gegebenenfalls die zu Hilfeleistung herangezogenen Institutionen vollständig angegeben habe.

Die Dissertation wurde nicht schon als Masterarbeit, Diplomarbeit oder andere Prüfungsarbeit verwendet.

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Veröffentlichungen in wissenschaftlichen Zeitschriften

1. Farahani F, **Korehi H.**, Phytoplankton diversity and nutrients at Jajerood River. Pakistan Journal of Biological Sciences. 9(9) (2006) 1787-1790.

2. **Korehi H**., Kock D., Dold B. and Schippers A., Occurrence of acidophilic halotolerant Fe(II)-oxidizing microorganisms in a marine shore mine tailings deposit at Chañaral, Atacama desert, northern Chile. In: Proceedings of the 19th International Biohydrometallurgy Symposium, Changsa, China. (2011) 931-935.

3. **Korehi H.**, Blöthe M., Stinikova M.A., Dold B. and Schippers A., Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailing in the Atacama Desert, Chile. Environmental Science and Technology 47 (2013) 2189-2196.

4. **Korehi H.** and Schippers A., Bioleaching of a marine hydrothermal sulfide ore with mesophiles, moderate-thermophiles and thermophiles. Advanced Materials Research 825 (2013) 229-232.

5. **Korehi H.**, Blöthe M. and Schippers A., Microbial diversity at the moderate acidic stage in three different sulfidic mine tailings dumps generating acid mine drainage. Research in Microbiology 165 (2014) 713-718.

Vorträge

1. **Korehi H.**, Mazaheri-Asadi M. and Nejatkhah-Manavi P., Effect of environmental changes on abundance of cyanobacteria in Tiab Estuary, Persian Gulf. 42th European Marine Biology Symposium, Kiel, Germany, 2007.

2. **Korehi H.**, Kock D., Dold B. and Schippers A., Occurrence of acidophilic halotolerant Fe(II)-oxidizing microorganisms in a marine shore mine tailings deposit at Chañaral, Atacama Desert, Northern Chile. National Symposium of Copper, Kerman, Iran, 2011.

3. **Korehi H.**, Kock D., Dold B. and Schippers A., Occurrence of acidophilic halotolerant Fe(II)-oxidizing microorganisms in a marine shore mine tailings deposit at Chañaral, Atacama Desert, Northern Chile.19th International Biohydrometallurgy Symposium (IBS), Changsha, China, 2011.

4. **Korehi H.**, Blöthe M., Sitnikova M.A., Kock D., Dold B. and Schippers A., Microbe-mineral interaction in an extreme arid and high saline mine tailings environment in northern Chile. Geo-Hannover, Hannover, Germany, 2012.

5. **Korehi H.**, Blöthe M., Stinikova M.A., Dold B. and Schippers A., Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile. Goldschmidt, Florence, Italy, 2013.

6. **Korehi H.**, Blöthe M., Stinikova M.A., Dold B. and Schippers A., Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile. 20th International Biohydrometallurgy Symposium (IBS), Antofagasta, Chile, 2013.

Poster

1. Farahani F., **Korehi H.,** A study in the seasonal variation of phytoplankton in Jajerood River. 14th National and the Second International Conference of Biology, Tarbiat Modares University, Tehran, Iran, 2006.

2. Farahani F., **Korehi H.,** Identification of cyanobacteria in Jajerood River and study of relationship with changing of nutrients materials. 6th Iranian Congress of Marine Science and the First Congress of Hydrography, Tehran, Iran, 2006.

3. **Korehi H.**, Mazaheri-Asadi M., Nejatkhah-Manavi P., Identification and study of monthly fluctuation of cyanobacteria in coastal waters of Bandar Abbas. 6th Iranian Congress of Marine Science and the first Congress of Hydrography, Tehran, Iran, 2006.

4. **Korehi H.**, Mazaheri-Asadi M., Nejatkhah-Manavi P., The influence of temperature, salinity and nutrients on cyanobacterial density in coastal waters of Bandar Abbas, Persian Gulf. 60th Annual meeting with the Northwest Algal Symposium, the Physiological Society of America, Juneau, Alaska, 2006.

5. **Korehi H.**, Mazaheri-Asadi M., Nejatkhah-Manavi P., Seasonal distribution of cyanobacteria in Khamir Port, Persian Gulf. 58th Annual Meeting of the Pacific Fisheries Technologists, Hermosillo, Sonora, Mexico, 2007.

6. **Korehi H.**, Wright T., Breuker A. and Schippers A., Some properties of acidophilic iron oxidizing bacteria enriched from acid mine drainage generating mine waste. Annual Conference of the Association for General and Applied Microbiology (VAAM), Bochum, Germany, 2009.

7. **Korehi H.**, Kock D., Dold B. and Schippers A., Geomicrobiology of high saline mine tailings in the Desert Atacama, Chile. Annual Conference of the Association for General and Applied Microbiology (VAAM), Hannover, Germany, 2010.

8. **Korehi H.**, Kock D., Dold B. and Schippers A., Detection and quantification of microorganisms in high saline mine tailings and two different open pits in the Desert Atacama, Chile. 13th International Symposium on Microbial Ecology (ISME), USA, Seattle, 2010.

9. **Korehi H.**, Kock D., Dold B. and Schippers A., Occurrence of acidophilic and halotolerant Fe(II)-oxidizing microorganisms in high saline mine tailings from the Atacama Desert, Chile. Annual Conference of the Association for General and Applied Microbiology (VAAM), Karlsruhe, Germany, 2011.

10. **KorehiH.**, Kock D., Dold B. and Schippers A., Occurrence of acidophilic and halotolerant Fe(II)-oxidizing microorganisms in high saline mine tailings from the Atacama Desert, Chile. 8th International Symposium of Subsurface Microbiology (ISSM), Garmisch- Partenkirchen, Germany, 2011.

11. **Korehi H.**, Blöthe M., Sitnikova M.A., Kock D., Dold B. and Schippers A., Microbe-mineral interaction in an extreme arid and high saline mine tailings environment in Northern Chile. 14th International Symposium on Microbial Ecology, Copenhagen, Denmark, 2012.

12. **Korehi H.**, Blöthe M., and Schippers A., Microbial diversity in four different sulfidic mine tailings. 20th International Biohydrometallurgy Symposium (IBS), Antofagasta, Chile, 2013.

13. **Korehi H.**, and Schippers A., Bioleaching of marine hydrothermal sulfides with mesophiles, moderate- thermophiles and thermophiles. 20th International Biohydrometallurgy Symposium (IBS), Antofagasta, Chile, 2013.