1 Do microbes control the formation of giant copper
2 deposits?

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13 **ABSTRACT**

14 The formation of secondary copper deposits, the source of more than half of the
15 world's production, is usually interpreted as abiogenic. In this study of the Las Cruces
16 deposit (SW Spain), in-situ hybridization and SEM analysis, together with integrated
17 genomic and bioinformatic studies on cultures, provide compelling evidence that a
18 microbial community controls the nowadays formation of the secondary copper
19 mineralization. The cementation zone of this deposit contains abundant microbial life
20 dominated by sulfate-reducing bacteria that coexist with methanogens and with other
21 prokaryotes having unknown roles. Fractures in the primary massive sulfides are coated
22 by extracellular polymeric substances in which the microbial cells are embedded.

Covellite crystals have nucleated within these microbial aggregates, accreting and forming large crystals attached to the vein walls. These results strongly suggest that in situ microbial sulfate reduction can control the formation of secondary copper deposits. Equivalent processes could be widespread in similar deposits elsewhere, but are probably overlooked due to the presumed low capability for fossilization of the microbes.

INTRODUCTION

In the deep biosphere, microbial communities coexist in complex and poorly known ecosystems where microorganisms play a major role in the solubility and precipitation of minerals (e.g., Fyfe, 1996). Of special scientific and economic interest is the role of these microbial communities in ore-forming processes. Being major controllers of sulfate reduction, iron-redox reactions, and the oxidation of organic matter, microorganisms can exert a major influence on the formation of different types of mineralization including exhalative massive sulfides, banded iron formations, and secondary metal ores (e.g., Nordstrom and Southam, 1997; Fallick et al., 2001; Konhauser et al., 2002).

Zones of cementation are formed below zones where pyrite-rich deposits are affected by subaerial oxidative weathering. Dissolution of pyrite takes place via oxidation of the reduced sulfur to sulfate (Nordstrom and Southam, 1997; Mielke et al., 2003), a process catalyzed by microbial activity that can speed up by ca. 10^5 times the abiogenic dissolution of pyrite (Singer and Stumm, 1970). The dissolution of pyrite leads to the formation of acidic waters that, in turn, dissolve other accompanying sulfides, leaving behind a Fe-rich gossan. Resulting metal-rich waters percolate downwards until they reach the oxygen-poor water table. Copper can precipitate here owing to changes in redox

state, forming a cementation zone enriched in chalcocite or covellite (Anderson, 1955; Sillitoe, 2005).

Despite a consensus that the supergene oxidation of pyrite is controlled by microbial processes, current models assume that formation of the cementation zone is predominantly abiogenic. This inference is based on the fact that the neoformed sulfides have similar sulfur isotope values to those of the replaced pyrite (Field and Gustafson, 1976). Alpers and Brimhall (1989) suggested that bacterial activity likely controlled the formation of supergene copper ore in the giant La Escondida deposit in Chile. Sillitoe et al. (1996) described small (0.02–0.5 μm) structures resembling bacteria in several secondary copper deposits, and proposed that the sulfides precipitated by microbial fixation of copper. However, Enders et al. (2006) argued that the morphologies described by Sillitoe et al. (1996) were not biogenic. Importantly, the latter authors detected only small amounts (10^3 cells/g) of sulfate-reducing bacteria in cultures grown from the cementation zone of the Morenci (Arizona) deposit, concluding that any biogenic contribution to the formation of secondary copper mineralization in that deposit is negligible. Other studies have postulated, on indirect evidence from sulfur isotopes, a biogenic influence in the formation of secondary copper, gold, silver, and zinc deposits (Melchiorre and Williams, 2001; Bawden et al., 2003; Rainbow et al., 2006).

THE LAS CRUCES DEPOSIT

The Las Cruces copper deposit, in the Iberian Pyrite Belt of southwestern Spain, hosts one of the richest cementation zones worldwide, containing ca. 16 Mt of high-grade (7%) copper ore (Fig. 1). Secondary mineralization formed during denudation and oxidation of a volcanogenic massive sulfide orebody of Late Devonian age (Tornos et al.,

2013; Yesares et al., 2014; Tornos et al., 2017). The secondary ore includes a low-grade cementation zone in which the secondary copper-rich minerals replace the massive sulfides, and more restricted fault-related veins containing massive chalcocite or covellite (Figs. DR1 and DR2). Since ca. 85 Ma, acidic descending waters derived from oxidation of the outcropping massive sulfides mixed with alkaline, anoxic, and hot waters that flowed upward along faults (Tornos et al., 2017). Deposition during the Messinian of an unconformable layer of marl changed the hydrology of the system. Thereafter, the orebody was isolated from the atmosphere and the unconformity between the Paleozoic basement and the Neogene cover channelized a confined aquifer that hosts alkaline, SO_4 -rich bicarbonate waters that currently mix in the mine with waters upflowing along the faults. The temperature of secondary mineralization has fluctuated between ca. 30–40 °C and 100–120 °C (Tornos et al., 2017). The cementation zone is capped by a rock composed of carbonates, galena, and iron sulfides that has been attributed previously to biogenic modification of a former gossan below the sealing marl (Tornos et al., 2014). This rock is rich in Fe, Pb, Ag, Au, Sb, and Hg, in marked contrast with the abundance of Cu-rich sulfides in the underlying cementation zone.

RESULTS

Selected samples of the cementation zone, composed of pyrite with fractures infilled by covellite (Fig. DR1), were obtained from short drill cores sited in the bottom of the Las Cruces open pit (see Supplementary Information). CARD-FISH methods have detected and quantified the presence of aggregates of microorganisms having specific metabolic activities. These aggregates are enclosed by self-generated, extracellular polymeric substances (EPS) that are attached to the cavity walls (Fig. 2B, 2F; Fig DR3B,

DR3F, and DR3J) and harbor different taxonomic groups. The probes have detected *Crenarchaeota* (Fig. DR3C), bacteria of the genera *Desulfitobacterium*, *Desulfosporosinus*, and *Dehalobacter* (Fig. 3C), sulfate-reducing bacteria (SRB) belonging to the order Delta-Proteobacteria (Fig. 2G), and the methanogenic *Methanosarcinales* archaea (Fig. DR3F). DAPI stained all of the microbial aggregates hybridized with CARD-FISH probes. The detection of DAPI staining with the absence of a CARD-FISH signal in parts of the microbial aggregates is consistent with the presence of mixed microbial populations.

Estimates (Supplementary Information) suggest that these samples host a high number of microbial cells, ca. $4.1 \pm 0.2 \times 10^7$ cells/g rock for bacteria and $6.3 \pm 0.6 \times 10^7$ cells/g rock for archaea. These microbial estimations are three to four orders of magnitude greater than those detected by Enders et al. (2006) in the cementation zone of the Morenci copper deposit.

FE-SEM images show that voids and fractures within the ore host abundant putative microbial aggregates that can infill the entire cavity (Figs. 3 and DR4). Coccoid-shaped structures <500 nm in diameter form groups of similar size, morphology, and distribution to the microbial aggregates detected by CARD-FISH, and are likely imbibed in EPS (Figs. 3A and B and DR3B). SEM images show that abundant euhedral plates of covellite ca. 0.5–1 μ m in diameter are growing directly on the EPS matrix (Figs. 3A to C); these plates coalesce on edges of the cavities, forming massive polycrystalline aggregates up to several mm in size that constitute the bulk of the vein filling (Figs. 3 and DR4). Co-localization in the EDS maps of Cu and Os, the latter highlighting the presence of organic compounds due to selective staining by OsO₄ of the lipids (Wierzbos and

Ascaso, 1994), confirms the close association between the microbial aggregates and covellite precipitates (Fig. DR4D). No covellite plates have been found outside of the microbial aggregates.

Anaerobic enrichment cultures are consistent with the results above. Cultures have released large amounts of H₂ gas (1–2.5 µmol/g rock) and low, but pervasive, methane (up to 7 nmol/g rock), while the metabolic activity consumes large amounts of sulfate (25–30 µmol/g rock). Both low CH₄ production and high sulfate consumption are consistent with widespread microbial sulfate reduction. DNA extraction, PCR amplification, and pyrosequencing of the cultured samples confirm the CARD-FISH data, and reveal the presence of Crenarchaeota, sulfate-reducing bacteria such as *Desulfosporosinus*, *Desulfitobacterium*, *Desulfotomaculum*, and *Desulfovibrio* sp., as well as archaea of the Methanosarcinales Order such as *Methanosarcina*. Cultures also suggest that these microbes are accompanied by lesser amounts of other microorganisms, including those specialized in the iron cycle, as well as by many other undetermined microorganisms having unknown roles (Table DR1) that potentially are responsible for generating the large amounts of observed H₂ gas.

DISCUSSION

The primary Paleozoic massive sulfides that host the cementation zone at Las Cruces have δ³⁴S values of ca. –6.8 to +10.3‰. These values differ from those of the high-grade copper sulfide veins (–2.4 to +21.7‰), indicating that replacement of the primary sulfide assemblage is not the sole mechanism for forming the secondary mineralization. The δ³⁴S compositions tend to be as high as that of the aqueous sulfate within the modern aquifers (–17 to +21‰), a result attributed to biogenic reduction in a

system with an excess of electron donors (Tornos et al., 2014). If sulfur is not inherited from the host sulfides and the descending fluids are Fe- and Cu-bearing and oxidized, iron, copper, and sulfate reduction are necessary for sulfide formation. At the temperatures of formation, below ca. 100–120 °C, and at pH >4, abiotic reduction of aqueous sulfate is kinetically inhibited (Ohmoto and Lasaga, 1982) and microbial sulfate reduction is the only known mechanism able to produce significant amounts of $\text{H}_2\text{S}_{\text{aq}}$ (Machel, 2001).

The direct relationship observed between microbial aggregates and covellite in the cementation zone of the Las Cruces deposit and the in situ detection of abundant sulfate-reducing bacteria strongly suggest that the current formation of the high-grade copper mineralization is controlled by microbial metabolism, with the covellite precipitating due to the increase of microbially mediated reduced sulfur. Precipitation of the copper sulfides also requires aqueous copper to be reduced from Cu^{2+} to Cu^+ , a process that could be biologically mediated by some chemolithotrophic gammaproteobacteria related to *Acidithiobacillus ferrooxidans* at low pH under aerobic conditions (Johnson et al., 2017). Alternatively, in anaerobic environments, aqueous Fe^{2+} is a rapid abiogenic reductant for copper under highly variable conditions (Matocha et al., 2005). In this model, a concomitant rise in aFe^{2+} would thus be responsible for the precipitation of secondary pyrite, bornite, and chalcopyrite.

The relationship of the sulfides with the EPS is considered a product of the adaptive strategy in lithobiotic communities (Flemming et al., 2007) that aids in nucleation of the sulfides. Here, glycoconjugates provide significant amounts of sorption

160 sites for nutrients, heavy metals, and water, keeping all of these components available for
161 microbial metabolism (Watnick and Kolter, 2000).

162 Methanogenic archaea have been detected in both the CARD-FISH analyses and
163 enrichment cultures (Table DR1). These archaea are ubiquitous in different types of
164 subsurface habitats (Kotelnikova, 2002) and in submarine-hydrothermal vents (Teske et
165 al., 2002; Nercessian et al., 2005). However, methanogens and sulfate-reducing bacteria
166 are not thought to share the same environment because they compete for the same
167 electron donors. The co-existence of these likely competing metabolic activities can be
168 explained by the presence of different environmental micro-niches within the solid rock
169 matrix.

170 Factors that currently stimulate microbial life in the Las Cruces deposit include
171 relatively high temperatures of up to 100–120 °C (Tornos et al., 2017) that can increase
172 the metabolic rate of some sulfate reducers (Stetter, 1996) and location of the
173 mineralization within a zone of fluid mixing having large redox and chemical gradients.
174 But what appears to mainly control metabolic activity of the microorganisms and copper
175 ore formation is the supply of large quantities of electron donors. Oxidation of H₂ is the
176 primary energy source within these environments (Nealson et al., 2005) and hydrogen is
177 thought to be released, together with methane and hydrocarbons, during the diagenesis of
178 underlying organic matter-bearing metasedimentary rocks that are widespread throughout
179 the Iberian Pyrite Belt (Tornos et al., 2008; Sáez et al., 2011). The faults that control the
180 Tertiary basin and extent of the cementation zone are pathways for upflowing waters,
181 and, likely also for gas that accumulates beneath the sealing marl. In fact, sediments of

the Guadalquivir basin host significant amounts of dry gas (Meléndez-Hevia and Alvarez del Buergo, 1996).

CONCLUSIONS

The presence in the Las Cruces deposit of metabolically active anaerobic prokaryotes coexisting with precipitating covellite reflects an active subsurface ecosystem that promotes and controls the precipitation today of copper sulfides. Results presented here also suggest that the geomicrobiological cycles involved in secondary ore genesis are probably not unique to Las Cruces, thus strongly suggesting that similar processes may be responsible for forming many other secondary copper deposits worldwide. However, these ecosystems are foreseen to be much more complex than geologic studies alone can predict, because most products of the microbial metabolisms do not precipitate minerals, and presently available techniques do not readily allow the tracking of their existence and complexity.

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FIGURE CAPTIONS

Figure 1. Geological map showing the location of Las Cruces copper deposit. Dotted lines are inferred Alpine faults below Tertiary sedimentary cover. UTM (Zone 29S). Modified from Tornos et al. (2017).

Figure 2. Representative micrographs of hybridized samples obtained by CARD-FISH with different oligonucleotides probes. Sample LC-5. Blue signal (A and E) shows microbial DNA stained with DAPI. Gray and white signals highlight covellite matrix. B and F signal (green) shows glucose/mannose residues of microbial EPS stained with FITC-ConA. Red signal (AlexaFluor-594-Tyr) corresponds to hybridization signals obtained by different oligonucleotide probes: (C) *Desulfitobacterium* and *Desulfosporosinus* microorganisms with DSP468 probe; (G) Sulfate-reducing bacteria from Deltaproteobacteria class targeted with SRB385 probe. (D) and (H) show merged image of three previous channels. Scale bars represent 5 μ m in all cases.

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342 Figure 3. SEM image of fracture in pyrite-rich massive sulfide showing covellite closely
343 associated and likely coeval with microbial cells. Sample LC-5. (a) Early covellite (cv I)
344 forms platy euhedral crystals coalesced into larger grains (cv II) that occur on selvages of
345 the vein. (b) FE-SEM image showing microbial aggregates including minute euhedral
346 plates of covellite (cv) embedded in EPS matrix that bind microbial aggregates to cavity
347 walls. (c) FE-SEM image of microbial cells intergrown with minute euhedral plates of
348 covellite (cv). Arrows denote microbial cells.

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350 1GSA Data Repository item 2018xxx, methods and complementary figures and tables, is
351 available online at <http://www.geosociety.org/datarepository/2018/>, or on request from
352 editing@geosociety.org.

37°35'N

Los Frailes

Aznaalcóllar

Gerena

La

DAPI Staining

FITC - ConA Staining

Probe stained with
AlexaFlour-594

Merged image

DSP468

SRB385