

Geochemical Modeling of ZnS in Biofilms: An Example of Ore Depositional Processes

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Abstract

The precipitation of nearly pure, nanocrystalline zinc sulfides (primarily sphalerite and wurtzite) within a biofilm dominated by sulfate-reducing bacteria of the family *Desulfobacteriaceae* has been observed in the flooded tunnels of an abandoned mine in southwestern Wisconsin. ZnS accumulations are limited to biofilms growing on old mine timbers. ZnS deposits, which comprise about 20 percent of the volume of the biofilm, formed in less than 30 yr from solutions containing only a few milligrams per liter (mg/l) Zn^{2+} . A model is proposed wherein sulfate reduction is followed by the formation of aqueous metal-sulfide clusters that aggregate to form 1- to 3-nm-diameter crystals that are transported and agglomerate to form micron-scale aggregates. Geochemical modeling shows how reduction of sulfate leads to exclusive precipitation of ZnS until most of the Zn^{2+} is consumed. The model also predicts a series of discrete metal-sulfide precipitation events that may be used to interpret sulfide mineral paragenesis of low-temperature Cu-Pb-Zn ore deposits. For example, the paragenesis of essentially monomineralic and mixed sulfide layers in Mississippi Valley-type, strataform, and strata-bound Pb-Zn-Cu, and SEDEX deposits can be predicted thermodynamically if the rate of aqueous sulfide generation does not outstrip the flux of metals into the system. Based on the characteristics of ZnS produced by sulfate-reducing bacteria, features of ore deposit minerals consistent with a biogenic origin can be identified. These include microstructures formed by coarsening of nanophase materials, the presence of micron-scale spherical aggregates in organic-rich microenvironments, and spatially heterogeneous patterns of sulfide mineral distribution, consistent with local variations in redox potential and activity of sulfate-reducing bacteria.

Introduction

THE ORIGIN of many low-temperature, metal-sulfide ore deposits has been the topic of numerous prior studies (Siebenthal, 1915; Bastin, 1926; Sangemeshwar and Barnes, 1983; Haynes, 1986; Sverjensky, 1986; Spirakis and Heyl, 1995; Large et al., 1998; Garven et al., 1999). Given the dramatic inhibition of thermochemical sulfate reduction at low temperatures, the potential role of microorganisms in sulfide mineralization has been proposed. Siebenthal (1915) suggested that microbial sulfate reduction contributed to the formation of strata-bound zinc sulfide deposits, and Bastin (1926) proposed a biogenic role in the formation of Mississippi Valley-type deposits. Trudinger et al. (1972) reviewed the feasibility of biogenic ore formation and addressed three issues: (1) the environmental limits of biogenic sulfate reduction; (2) whether the age of biological sulfate reduction was coextensive with the ages of ancient sulfide deposits; (3) whether the rates of sulfate reduction are sufficient for ore formation. Trudinger et al. (1972) concluded that the information at the time was not adequate to address these questions. In the last 30 yr, significant progress has been made on each of these issues.

Recently, we obtained direct evidence for precipitation of concentrated deposits of ore minerals by microorganisms. Labrenz et al. (2000) reported that spherical aggregates of nanocrystalline ZnS constitute about 20 percent of the volume of white biofilms

dominated by sulfate-reducing bacteria. The biofilms are growing in fairly dilute ground water (~1–5 ppm Zn) in abandoned tunnels of the Piquette mine in southwest Wisconsin. The ZnS aggregates contain minor amounts of Fe (0.2 wt %), Se (0.004 wt %), and As (0.01 wt %; Labrenz et al. 2000).

In this paper, we expand our initial brief report of bacterially mediated ZnS precipitation in subsurface regions adjacent to the Piquette mine in southwest Wisconsin. Specifically, we develop a geochemical model to describe the chemical evolution of ground water in this system that illustrates controls on metal transport and sequential deposition of sulfide minerals. Here, we analyze the thermodynamic and kinetic constraints on sulfate reduction and sulfide precipitation at low temperature and consider the relevance of our geochemical model to the interpretation of mineral paragenetic sequences during formation of ore deposits in the geologic record. Although the model chemistry is strictly independent of microbial metabolism, conceptual analysis of the model with kinetic parameters and known environmental limits on microbial activity also illustrates the potential role of microorganisms in low-temperature sulfide precipitation.

Field Site and Methods

Field site

The Piquette No. 1 mine is located in southwest Wisconsin, near the town of Tennyson. The mine is in the Potosi subdistrict, part of the Upper Mississippi Valley zinc-lead district (Sicree and Barnes, 1996). The Piquette No. 1 mine includes the Wilson workings, where biofilm samples were collected for this study.

Ore in the Piquette No. 1 mine is located within the lower beds of the Galena Dolomite and the upper beds of the Decora formation (Middle Ordovician), with veins of sphalerite

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and galena typically 1 m thick, but reported to be up to 13 m thick within the area (Heyl et al. 1959). Mines in the Potosi subdistrict contain lead and zinc primary sulfide minerals with associated pyrite, chalcopyrite, marcasite, and digenite hosted in carbonates and sulfates (Whitlow and West, 1966). The Zn/Pb ratio in the Upper Mississippi Valley district is approximately 40, with several episodes of earlier sphalerite deposition followed by increased galena deposition in the early Permian (Sicree and Barnes, 1996). Enargite and erythrite (copper sulpharsenide and cobalt arsenate, respectively) have both been reported to be associated with sphalerite in the Piquette No. 2 mine (Heyl, 1964). The mine ceased operation in 1968, at which time the tunnels were allowed to flood; the majority of the underground workings remain just beneath the local water table.

Sample collection

Samples were collected by highly trained and experienced technical SCUBA divers about 400 m from the entrance to the flooded mine. Water samples were collected above and next to the white biofilm sampling sites. Empty 500-ml Nalgene® bottles were carried by the divers to each of these sites, opened, and immediately capped after they were filled. After the samples were returned to the surface about 30 minutes later, aliquots were taken to measure pH, conductivity, alkalinity, and HS^- in the field. pH measurements were made with an Orion Model 290 portable meter with an Orion triode electrode calibrated with Fischer brand 4.0, 7.0, and 10.0 pH buffers. Alkalinity titrations were carried out with a Hach digital titrator using 1.600 N H_2SO_4 and a bromocresol Green/Methyl Red indicator protocol after USGS standard methods (Radtke et al., 1998). Samples for major cation and trace-metal analysis were filtered with a 0.2- μm Acrodisc filter and acidified to 1 percent v/v with Fischer-brand, trace-metal-grade hydrochloric acid. Samples for anion analysis were filtered with a 0.2- μm Acrodisc filter. All samples for laboratory analysis were kept on ice for transport and in a 4°C refrigerator in the laboratory until analysis was carried out within one week. Major cations (K^+ , Na^+ , Mg^{2+} , Ca^{2+}) were analyzed on a Dionex series 500 Ion Chromatograph using a 4-mm Dionex IonPac CS12A column under an isocratic 22-mM sulfuric acid eluent at 1.2 ml/min. Anions (Cl^- , NO_3^- , SO_4^{2-}) were analyzed by Ion Chromatograph with a 4-mm Dionex IonPac AS14A column using an isocratic sodium carbonate/bicarbonate eluent (at 1.5 ml/min). Trace metals were analyzed by ICP-MS (VG PlamsaQuad 3), operated in peak jumping mode. All standards, blanks, and samples were prepared in ultra pure (distilled), 2 percent HNO_3 . Multiple internal standards were used to correct for machine drift that was less than 2 percent across the mass range.

Electron microscopy

Sediment and biofilm samples were characterized by transmission electron microscopy (TEM) and energy-dispersive X-ray (EDX) microanalysis. TEM images, EDX spectra, and selected-area electron diffraction patterns were collected using a Philips CM200UT TEM operated at 200 kV and a JEOL JEM 1010 TEM operated at 80 kV. Samples were prepared for TEM characterization via standard impregnation and ultramicrotomy methods (see Hugenholz et al., 2001).

Geochemical modeling

The commercial geochemical modeling package Geochemist's Workbench, version 3.1, was used for all forward modeling (Bethke, 2000). The Lawrence Livermore National Labs V8 R6+ "combined" thermodynamic dataset was utilized for all calculations. Additional thermodynamic data for the phase mackinawite (FeS) was added to the database and pyrite precipitation was suppressed, in agreement with crystalline iron sulfide precipitation at these conditions (Schoonen and Barnes, 1991; Benning et al., 2000). The Log K values utilized for Mackinawite dissolution ($\text{FeS} + \text{H}^+ \rightarrow \text{Fe}^{2+} + \text{HS}^-$) are: 3.71 (10°C), 3.21 (25°C), 2.47 (50°C), and 1.29 (100°C; Benning et al., 2000). All models calculated were performed at 1 bar pressure.

Due to the increased contribution of surface energy to the total energy of the nanocrystalline materials (Zhang and Banfield, 1998), thermodynamic data obtained for bulk or microcrystalline phases will be different from the thermodynamic constants for nanocrystalline materials. This renders the smaller particle more soluble, the degree of which may be approximated by considering the interfacial tension, molecular volume, and particle radius (Borg and Dienes, 1992). Solubility may be correlated to surface tension (Söhnel, 1982) and, therefore, more insoluble minerals such as sulfides may have a greater contribution to total free energy due to smaller particle size. Although the literature is currently lacking sufficient definition of thermodynamic data corresponding to nanocrystalline metal sulfides, analysis of model results in this work indicates that errors of several orders of magnitude will not vary the interpretations of this study.

Modeling the progression of reducing conditions within an anaerobic biofilm utilized three different approaches: an Eh slide, organic acid titration, and aqueous sulfide titration. An Eh slide refers to the sequential decrease in the equilibrium electropotential of the modeled system, where each redox couple in the model is adjusted at each step. At each step, the Eh is decreased a specified amount, redox conditions for all redox active species are recalculated, and minerals are precipitated if supersaturated. Although not strictly correct in terms of electron balance in a system, an Eh slide is an effective way of generically modeling the progression of reductive conditions. The Eh of a solution can be defined for any system through the Nernst equation governed by any redox couple in equilibrium. Because of the paucity of redox equilibrium in natural waters (Langmuir, 1997), only the qualitative trend between Eh and $\text{SO}_4^{2-}/\text{HS}^-$ is meaningful, which is the basis for the models presented here. Graphical representations depicting mineral and fluid chemistry changes as a function of electropotential, reacted organic substrate, or titrated $\text{H}_2\text{S}_{(\text{aq})}$ may be used to look at different aspects of the same system, and results from each of these 3 methods are essentially identical and interchangeable for all of the calculations presented. Model diagrams depicting the left-to-right progression of reducing conditions in any system indicate mineralogical and fluid chemical changes associated with precipitation/dissolution and redox-sensitive speciation reactions.

Controls on metal concentrations and transport mechanisms in ore solutions may differ from those encountered at the Piquette mine, due to differences in chloride content and

temperature. It is generally accepted that the ore-forming solutions are deep brines that contain upwards of 10 percent chloride (approximately 3 molal). Solutions at these ionic strengths stretch the limits of the extended Debye-Hückel (or B-dot) model, and appropriate virial metal complexation data does not exist (Bethke, 1996). Modeling of ore-forming solutions associated with SEDEX and Mississippi Valley-type deposits undertaken in this work are performed at concentrations of 0.1 and 3 molal sodium chloride (Table 3). The process of sulfate reduction and the sequential precipitation of metal sulfides from ore solutions in excess of 3 molal may still be qualitatively modeled by performing an Eh slide on the solution composition and assuming activity coefficients as calculated at a limit of 3 molal.

Results

Sample characterization

TEM-based characterization of whitish biofilms revealed that they contain abundant, micron-scale aggregates of few-nm-diameter ZnS crystals (Fig. 1). Sulfide aggregates are imaged as dark balls (fractures were introduced during microtome sample preparation). Rings in the inset selected-area electron diffraction pattern have *d*-values that correspond to sphalerite (though twinned sphalerite and wurtzite have also been detected; data not shown). The presence of rings in the selected-area electron diffraction pattern, and their fuzzy nature, indicates the aggregates are polycrystalline, and that the particle diameters are in the few- to few-tens-of-nm range.

The predominant microbial species responsible for ZnS formation are sulfate-reducing bacteria of the family *Desulfobacteriaceae* (Labrenz et al., 2000). These dissimilatory sulfate-reducing bacteria utilize sulfate as an electron acceptor for the oxidation of organic compounds. However, numerous other microbial taxa have been detected in the biofilm (Labrenz and Banfield, submitted). Consequently, we cannot be certain that the cells shown in Figures 1 and 2 are sulfate-reducing bacteria.

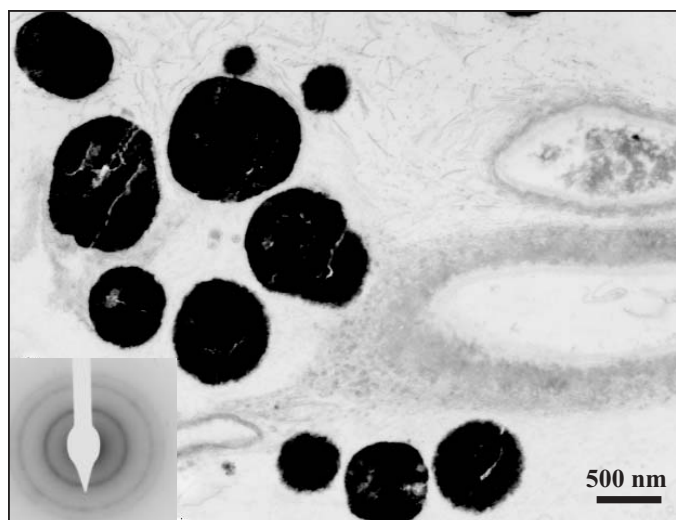


FIG. 1. TEM image of a microtomed slice of the white biofilm from the Piquette mine in southwestern Wisconsin. Round black aggregates are ZnS; note microbial cells on the right side of the image. Selected-area diffraction image of ZnS ball is shown at lower left.

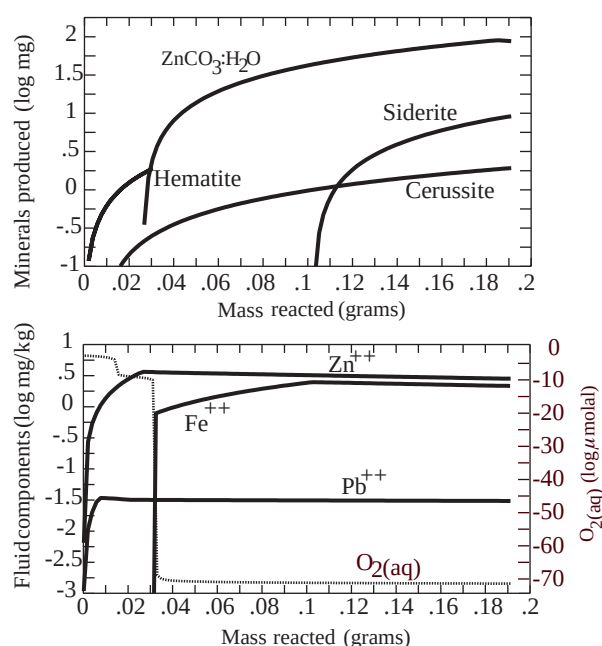


FIG. 2. GWB 3.1 React simulation of the oxidation of the original ore at the Piquette mine. Reacted ore contains: 40 mg sphalerite, 1 mg galena, 10 mg pyrite, and 140 mg dolomite in 1 l of water at 25°C with similar major element concentrations as solution presented in Table 3. Note Zn, Fe, Pb concentrations as the solution is reduced through the oxidation of the ore.

In the prior report of Labrenz et al. (2000), the origin of the biofilm was not discussed. Further examination of the site by the SCUBA team, in combination with extensive scanning electron microscope (SEM), TEM, and optical microscope characterization, indicates that the biofilms are growing on, and within, old mine timbers. The organic acids utilized by the sulfate-reducing bacteria may be provided by degradation of refractory organic compounds within the timbers by fermentative bacteria within the biofilm (Labrenz and Banfield, submitted).

Water chemistry reported in Table 1 was from the sample collected closest to the white biofilm and is the most complete water analysis with the lowest charge balance error determined at the site. Measured zinc concentrations from over 4 sampling trips to the Piquette mine in 1 yr were between 0.25 and 3.0 mg/l.

Geochemical modeling—Piquette mine

Three types of geochemical processes involved with the evolution of ground waters in the Piquette mine have been modeled. The oxidation of the original sulfide ore deposit by oxic ground waters and the reoxidation of that fluid, once inside the open channels of the flooded mine, describe the composition of the fluid sampled at the site. The reduction of sulfate in this solution through microbial activity is modeled to describe the geochemical evolution of the solution to form monomineralic sulfide precipitates. This model of how an oxic fluid evolves with progressive reduction is then used to look at other environments in which oxic fluids are reduced to form sulfide mineral deposits.

TABLE 1. Water Chemistry of Sample Collected near the White Biofilm Where Biogenic ZnS Precipitation Has Been Observed

Water chemistry in the Piquette mine near the white biofilm		units
Na ⁺	18.5	mg/l
K ⁺	1.8	mg/l
Mg ²⁺	61.3	mg/l
Ca ²⁺	105.8	mg/l
SO ₄ ²⁻	54.3	mg/l
Cl ⁻	49.6	mg/l
NO ₃ ⁻	1.4	mg/l
PO ₄ ³⁻	4.7	mg/l
HCO ₃ ⁻	480	mg/l
Cr	0.61	μg/l
Mn	22.5	μg/l
Co	1.48	μg/l
Ni	9.45	μg/l
Cu	7.82	μg/l
Zn	1362.5	μg/l
As	0.56	μg/l
Rb	0.74	μg/l
Sr	262.4	μg/l
Cd	2.23	μg/l
Ba	1110.9	μg/l
Pb	0.852	μg/l
U	1.04	μg/l
CBE	3.7%	

Abbreviations: CBE = charge balance error

Figure 2 depicts the results of modeling reactions between ZnS-PbS-FeS₂ ore (mineral ratio on wt % basis = 4/1/1) in dolomite host rock with a packet of ground water initially saturated with atmospheric O₂. Zinc solubility in the resulting solution is controlled by the formation of ZnCO₃ · H₂O, which limits the aqueous zinc concentration (as Zn²⁺) to approximately 2.5 mg/l (38 μM). Lead solubility is controlled by the precipitation of cerussite (PbCO₃) to a concentration of less than 1 μg/l (as PbCO_{3(aq)}). Aqueous iron concentration (1.8 mg/l Fe_T [32 μM] as FeHCO₃⁺ and Fe²⁺) is limited by the solubility of siderite under anaerobic solutions.

After oxidizing ground-water solutions react with the ore, they are transported away from the ore via fracture flow. Because the oxygen has been consumed by reaction with the ore, the sulfate-carbonate fluids are moderately reduced. Thus, they are able to transport considerable ferrous iron and zinc over long distances in the subsurface.

Field observations reveal that in regions where fractures intersect the mine tunnels, pronounced redox gradients develop. Large accumulations of biogenic Fe-oxyhydroxides (ferrihydrite and goethite) indicate mixing between ferrous iron-bearing sulfate solutions and more oxidized solutions (Banfield et al., 2000). The titration of the iron sulfate solution with aqueous O₂ is modeled in Figure 3. Note that while the iron concentration is dramatically decreased, the model does not predict changes in the zinc or lead concentrations.

In parallel work, it has been experimentally determined that ferrihydrite and goethite sorb and structurally incorporate approximately 4 percent (w/w) Zn and 0.05 percent (w/w) Pb (Fowle et al., in prep). The mass of ferric oxyhydroxide precipitated within the system, and the ground-water flow rate are unknown. However, if the iron concentration in the

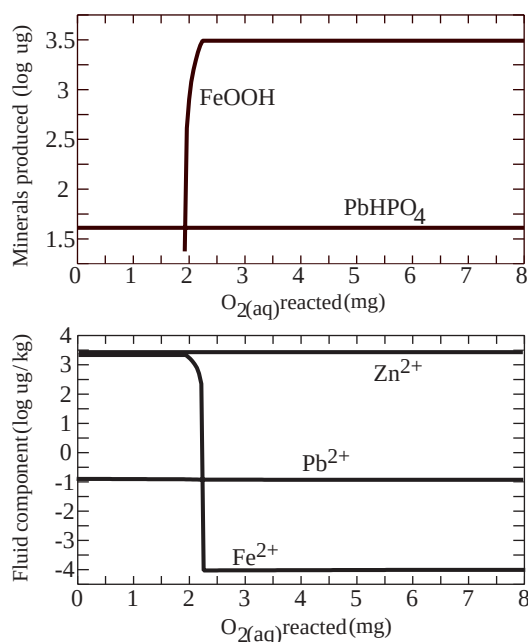


FIG. 3. GWB 3.1 React model of the oxidation of water from the Piquette mine after oxidation of primary ores and transport into the flooded tunnels. Calculation performed using aqueous solution output from Figure 3 calculation in a system open to air.

reduced fluid is 1.8 mg/l, limited by siderite solubility, and the iron concentration following fluid mixing is 1 ng/l, determined by ferrihydrite solubility, approximately 0.1 mg Zn and 4 ng Pb would be incorporated into ferric oxyhydroxide precipitating from 1 l of water. This reaction requires 2 mg O_{2(aq)} (Figure 3). The concentrations of Pb and Zn are relatively unaffected by sorption onto the oxyhydroxides and are maintained at approximately 2.5 mg/l and 0.5 μg/l, respectively.

The solution composition achieved by forward modeling, as described above, correlates well with the fluid composition measured within the mine tunnels (Table 1) in proximity to Fe-oxyhydroxide and white biofilms. Thus, we infer that this solution is appropriate for forward modeling of subsequent mineralization within the white biofilms.

The evolution of the tunnel solution (Table 1) with the decrease in Eh (reflecting an increase in aqueous sulfide concentration) is modeled in Figure 4. Following the onset of ZnS precipitation, the HS⁻ concentration is buffered until the majority of the Zn²⁺ is consumed, at which point HS⁻ activity continues to increase until the fluid becomes saturated with respect to the next sulfide, galena. Note that the thermodynamic model indicates a specific series of discrete sulfide precipitation events as the fluid becomes more reduced.

The line that predicts the precipitation of ZnS in Figure 4 is curved because the amount of HS⁻ to achieve saturation increases as the Zn²⁺ concentration decreases. If the sulfide concentration exceeds that required for saturation with respect to ZnS, other sulfides will precipitate. The observation of a monomineralic precipitate indicates that the rate of sulfide generation is coupled to the flux of Zn²⁺, such that ZnS precipitation effectively buffers HS⁻ concentration below galena saturation.

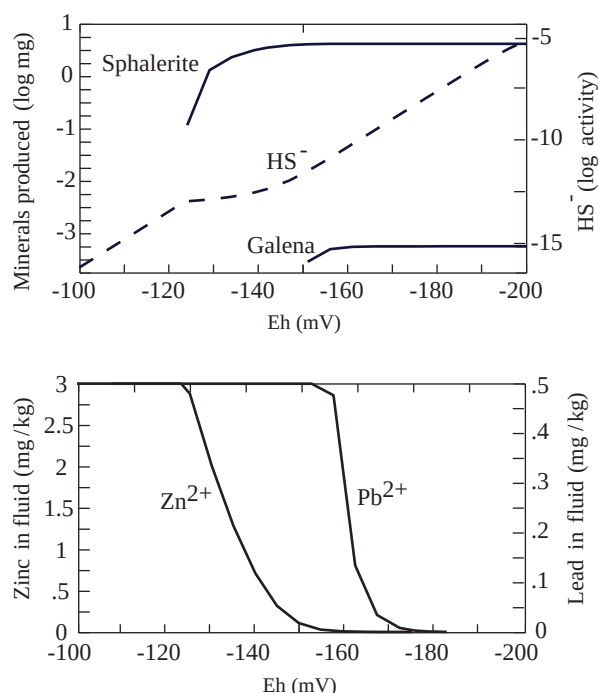


FIG. 4. GWB 3.1 React model of the reduction of water in the Piquette mine interacting with sulfate-reducing bacteria in the white biofilm. Calculation performed at 25°C.

At very small scales, HS^- accumulates within the biofilm until concentrations exceed that required for homogeneous nucleation of sphalerite. The αZn^{2+} in a Piquette mine solution is around 1.5×10^{-5} and, at supersaturated conditions ($\log \text{IAP}/K_{\text{eq}} = 1$), the αHS^- is predicted to be about 1×10^{-15} . As illustrated above, the metal concentration is significantly higher than the aqueous sulfide needed to achieve supersaturation. Thus, precipitation rates are presumably limited by the rate of production of sulfide, which is ultimately controlled by the supply of organic substrate that can be metabolized by sulfate-reducing bacteria. Nanocrystals either do not form in proximity to the cells or they are not prone to bind to functional groups on the cell surface, as cells are not coated by nanocrystals.

Discussion

Relevance in modern environments

The mineralization process documented in the Piquette biofilms (Labrenz et al., 2000) is relevant under a wide range of conditions, including, but not limited to, the range of conditions in which sulfate-reducing bacteria are found in the environment. The variety of habitats occupied by sulfate-reducing bacteria is defined by temperature, salinity, and availability of suitable organic substrates. The temperature range for sulfate-reducing bacteria survival spans the limit of known habitats for life. Organisms are able to reduce sulfate at temperatures in excess of 100° (below boiling due to hydrostatic pressures) in deep-sea hydrothermal vent sediments (Jorgensen et al., 1992), and in highly saline brine solutions (Table 2). These organisms are typically limited in the types of organic compounds they can use as organic energy sources.

TABLE 2. Environmental Limits of Some Known Sulfate-Reducing Bacteria Activities with Respect to Temperature and Salinity

Known environmental limits for selected sulfate-reducing bacteria species		
Sulfate-reducing bacteria	Temperature (Celsius)	Reference
<i>Thermodesulfobacterium hveragerdense</i>	55–74, opt. 70–74	1
<i>Thermodesulfobacterium islandicus</i>	45–70, opt. 65	1
<i>Thermodesulfobacterium norvegicus</i>	44–74, opt. 60	2
<i>Archaeoglobus veneficus</i> ¹	65–85, opt. 75–80	3
<i>Thermococcus</i> sp. DT1331 ¹	55–93, opt. 80	4
<i>Pyrococcus abyssi</i> ¹	67–102, opt. 96	5
NaCl-requirement (%)		
<i>Desulfocella halophila</i>	2–19, opt. 4–5	6
<i>Desulfotomaculum halophilum</i>	1–14, opt. 6	7
<i>Desulfovibrio vietnamensis</i>	opt. 5	8
<i>Desulfovibrio gabonensis</i>	Up to 17	9
<i>Desulfovibrio halophilus</i>	Up to 18	10
<i>Desulfohalobium retbaense</i>	opt. 10	11

Note: Temperatures and % NaCl indicate organism tolerance and optimum growth conditions (opt.);

¹ denotes the sulfate reducer is an archaea, not a bacteria

References: 1 = Sonne-Hansen and Ahring, 1999; 2 = Beeder et al., 1995; 3 = Huber et al., 1997; 4 = Kwak et al., 1995; 5 = Marteinsson et al., 1997; 6 = Brandt et al., 1999; 7 = Tardy-Jaquenod et al., 1998; 8 = Nga et al., 1996; 9 = Tardy-Jaquenod et al., 1996; 10 = Caumette et al., 1991; 11 = Ollivier et al., 1991

Substrates utilized are species dependent, and include H_2 , lactate, pyruvate, ethanol, fumarate, malate, choline, acetate, butyrate, benzoate, indole, propionate, and fatty acids (Brock and Madigan, 1991). These may be provided from a variety of sources, including fermentation associated with anaerobic microorganisms.

Coupling between sulfate reduction, mineralization, and microbial growth

Inorganic reactions between sulfate and organic compounds are kinetically inhibited at low temperatures. In the absence of an enzyme or other catalyst, these compounds will persist in disequilibrium for millions of years at these conditions (Ohmoto and Lasaga, 1982). The rapid formation of metal sulfide accumulations at ~8°C in the recently flooded Piquette mine implies the operation of an enzymatic pathway.

In order to facilitate enzymatic sulfate reduction, a sulfate-reducing bacterium imports the organic electron donors (organic compounds) and the electron acceptor (sulfate) inside the cell (see Fig. 5A). Acetate, for example, is oxidized to CO_2 within the cytoplasm through a modified citric acid cycle (Möller et al., 1987). Compounds such as NADH and FADH_2 transport electrons and protons from inside the cell (see Fig. 5B) to the cytoplasmic membrane (see Fig. 5C), where dehydrogenases separate electrons and H^+ . Protons are transported outside the membrane and electrons are passed to other membrane-bound enzymes with higher redox potentials. Exclusion of protons forms a proton gradient across the membrane, which drives the formation of ATP (see Fig. 5D), the principle currency of energy in cell functions. The

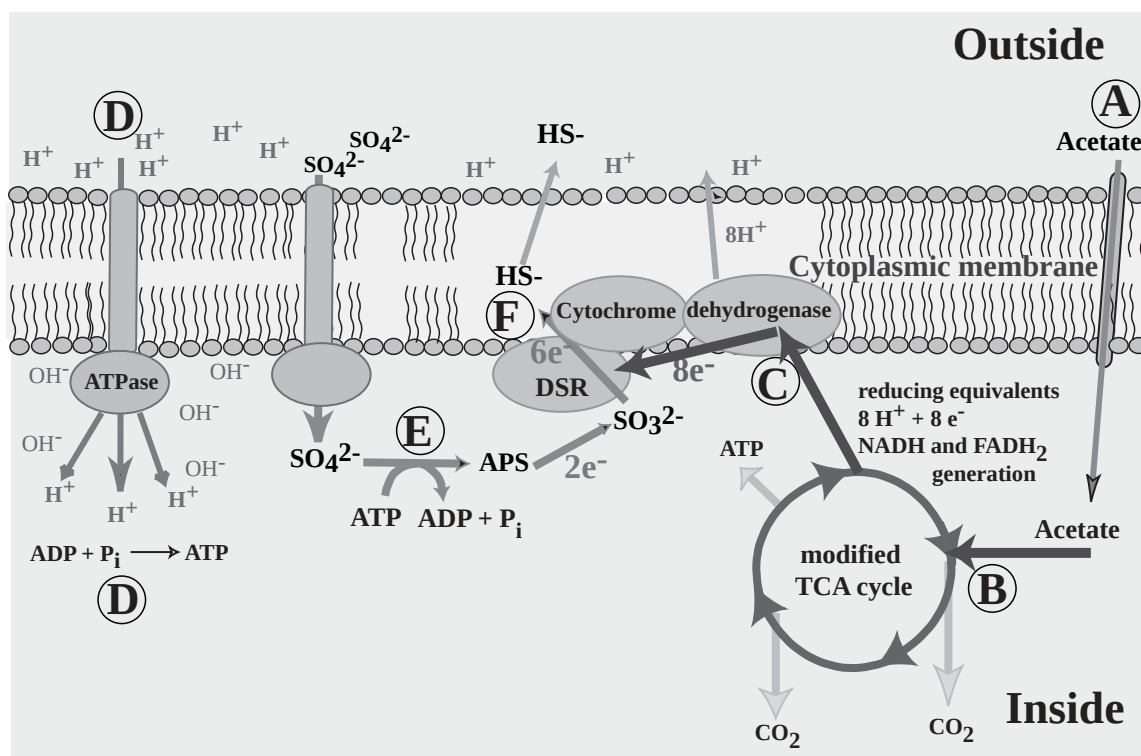


FIG. 5. Schematic representation of the metabolic processes of sulfate-reducing bacteria, illustrating the coupled oxidation of an organic substrate (acetate) with sulfate reduction. The respiration of H_2S is linked by this process with the generation of proton motive force across the membrane which drives ATP production. Modified from figures in Brock and Madigan (1991).

electrons are passed from these enzymes to adenine phosphosulfate, a sulfur-bearing molecule that is generated from sulfate (see Fig. 5E) and is reduced to sulfite. Although the exact mechanism of the complete reduction of sulfite to hydrogen sulfide is in dispute, reduction likely goes through trithionate and thiosulfate intermediates prior to formation of hydrogen sulfide (Chambers and Trudinger, 1979). H_2S is subsequently expelled through the cell wall and into solution (see Fig. 5F).

A high concentration of sulfide is actually toxic to microorganisms due to its ability to denature certain proteins and bind to metal-centered enzymes (Brock and Madigan, 1991). H_2S inhibits growth of sulfate-reducing bacteria cultures in the laboratory and it is assumed that the H_2S concentration will limit growth rates in closed systems (Postgate, 1965; Trudinger et al., 1972). The very low solubility of ZnS (and other metal sulfides) leads to precipitation reactions that limit the buildup of sulfide in solution, thus optimizing conditions for growth of sulfate-reducing bacteria.

Nanocrystal particle size, microstructure, and possible biosignatures

Crystal growth via Ostwald ripening is driven by surface free energy, which is effectively proportional to the density of undercoordinated surface ions (Zhang and Banfield, 1998). The persistence of 1- to 5-nm-diameter nanocrystals, despite the enormous driving force for crystal growth, suggests that subsequent crystal growth is inhibited by some mechanism.

One possible explanation is that uncoordinated surface ions are complexed by organic ligands. This strategy has been used to limit crystal growth in experimental ZnS syntheses (Torres-Martinez et al., 1999).

The ZnS crystals are commonly twinned on very fine scales and wurtzite is commonly present as intergrowths on {111} sphalerite (Moreau et al., 2001). Banfield suggests that these microstructures arise as the result of an aggregation-based crystal growth mechanism, probably commencing with clusters analogous to those described by Luther et al. (1999; also see Penn and Banfield, 1998, 1999). This observation is relevant to the current discussion because the existence of fine-scale twinning and structural intergrowths in coarsened sphalerite in ancient ore deposits may provide a useful biosignature (higher-temperature crystallization would not be expected to produce the very finely crystalline materials where this growth mechanism can predominate). Other potential biosignatures are discussed below.

Nucleation of ZnS

Luther et al. (1999) report evidence for tetrameric $\text{Zn}_4\text{S}_6(\text{H}_2\text{O})_4^{4+}$ clusters that are structurally analogous to sphalerite. Molecular modeling and spectroscopic data indicate that the diameter of this cluster is ~10 to 16 Å (Luther et al., 1999). Luther et al. (1999) suggest that these form an amorphous precipitate that would develop long-range ordering with further cross linking. However, the ZnS observed in the Piquette mine is not an "amorphous" precipitate. TEM lattice

TABLE 3. Selected Ore Fluid Compositions for Stratiform and Mississippi Valley-Type Deposits

Selected fluid chemistries used in modeling ore deposition			
	Average stratiform-deposit water composition ¹ [possible range]	low Cl- brine used in Figure 7A	high Cl- brine used in Figure 7B
Temp (°C)	25	50	50
pH	7.4–7.8, 3.5–6 ²	5.4	5.4
f_{O_2}	10^{-30} – 10^{-45} bars		
Na ⁺	3 [0.3–4.5]	0.1	3
K ⁺	0.1 [0.02–0.3]	0.1	0.1
Mg ²⁺	0.5 [0.01–1.7]	0.5	0.5
Ca ²⁺	0.1 [0.01–0.4]	0.1	0.1
SiO ₂		quartz solubility	quartz solubility
SO ₄ ²⁻	0.4 [0.01–2.0]	0.4	0.4
Cl ⁻	3 [0.05–6.0]	0.1	3
HCO ₃ ⁻	0.02 [0.001–0.1]	dolomite solubility	dolomite solubility
Fe _T (ppm)		10	10
Zn _T (ppm)		50	50
Pb _T (ppm)		0.6 (Cerussite solubility)	25 (Cerussite solubility)

Note: Concentrations in molality unless otherwise noted

¹ (Haynes, 1986)

² pH for metalliferous brines reported as 3.5 to 6 (Cooke et al., 2000)

fringe images show that the ZnS particles are crystalline, even though their diameters are often ≤ 2 nm. The diffraction peaks are broad, due to particle size effects, and structural characteristics may differ in detail from bulk crystalline material. While the exact structural state of nanoparticles is difficult to define, selected-area diffraction patterns are most similar to those from bulk sphalerite. However, high-resolution TEM, modeling of X-ray diffraction data, and synchrotron-based experiments indicate the presence of particles similar to both sphalerite and wurtzite (Moreau et al., 2001).

Zn₄S₆(H₂O)₄⁴⁻ should condense from neutral Zn₃S₃(H₂O)₆ rings with excess bisulfide, resulting in the formation of a sphaleritelike structure (Luther et al., 1999). Alternatively,

formation of a neutral Zn₆S₆(H₂O)₉ cluster from Zn₃S₃(H₂O)₆ rings would lead to a wurtzitelike structure. Sphalerite precipitation may be expected to predominate under biofilm conditions because anionic complexes dominate when the S/Zn ratio is >0.8 (Luther et al., 1996). In addition, at low temperature, wurtzite is considered to be unstable and should quickly transform to sphalerite (Daskalakis and Helz, 1997). However, verification that nanocrystalline sphalerite is the stable phase at low temperature and pressure requires inclusion of the surface energy contributions to the total free energy of both sphalerite and wurtzite (e.g., see Zhang and Banfield, 1998).

Relevance to ore deposit formation

The production of significant sulfide concentrations in ore-forming systems occurs through either thermochemical or biological pathways. Thermochemical sulfate reduction must occur at temperatures in excess of $\sim 175^\circ$ to 225°C to produce significant sulfide deposits (Ohmoto and Lasaga, 1982; Goldstein and Aizenshtat, 1994; Goldhaber and Orr, 1995), whereas below $\sim 120^\circ\text{C}$, biological sulfate reduction predominates (Jorgensen et al., 1992; Goldstein and Aizenshtat, 1994). It has been argued that thermochemical sulfate reduction may be a significant process down to temperatures of 100°C in sour-gas fields (Machel et al., 1995; Machel, 2001), but the application of even a general minimum temperature that low is currently unsupported in ore depositional settings.

The range of temperatures between those where thermochemical sulfate reduction and biological sulfate reduction dominate coincides with the temperatures inferred for formation of many deposits, including Mississippi Valley-type, SEDEX, and other stratiform/strata-bound deposits (Trudinger et al., 1985; Goldhaber and Orr, 1995; Machel et al., 1995; Sicree and Barnes, 1996; Ohmoto and Goldhaber, 1997). There may be a “dead zone” where sulfate reduction does not occur at an appreciable rate. Furthermore, as temperature and equilibrium conditions can fluctuate spatially and temporally (Barnes and Rose, 1998), ore formed by biological sulfate reduction and thermochemical sulfate reduction may exist within a single deposit.

Sicree and Barnes (1996) estimate a deposition rate of $0.2\ \mu\text{m}$ sulfide per year for Upper Mississippi Valley-type deposits. Considering a 1-m^2 area of $0.2\text{-}\mu\text{m}$ thickness and a molar volume of $23.83\ \text{cm}^3$ for sphalerite, we calculated that a sulfate reduction rate of $8.4\ \text{nmol SO}_4^{2-}\ \text{cm}^{-3}\ \text{a}^{-1}$ would be required. Uncatalyzed, thermochemical sulfate reduction at 125°C , pH 4, and $25\ \text{mM SO}_4^{2-}$ has been extrapolated to occur at a rate of approximately 10^{-3} to $10^{-4}\ \text{nmol SO}_4^{2-}\ \text{cm}^{-3}\ \text{a}^{-1}$ (Ohmoto and Goldhaber, 1997). Goldhaber and Orr (1995) showed a significant autocatalytic component to inorganic sulfate reduction coupled with toluene oxidation by the addition of H₂S. However, catalysis does not become significant until H₂S builds to a concentration that is several orders of magnitude more than required for metal sulfide equilibrium in circumneutral waters containing relatively low concentrations (tens of ppm) of metal. Another inorganic catalyst for this reaction at low temperature has not been discovered (Trudinger et al., 1985; Ohmoto and Goldhaber, 1997). The lack of an abiotic catalyst implicates biological activity as the most likely source of sulfide in low-temperature ore deposits.

A wide range of biological sulfate reduction rates has been measured in a variety of different natural environments (Trudinger et al., 1972; Skyring, 1987; Elsgaard et al., 1994), but even some of the slowest rates are well within the range required for sulfide deposition in Mississippi Valley-type deposits, as calculated above.

Are sulfate-reducing bacteria a plausible source of sulfide for ancient deposits? Sulfate-reducing bacteria can be traced back into the early Archean, 2900 to 3900 Ma (Vasconcelos and McKenzie, 2000). Investigating the phylogeny of dissimilatory sulfite reductase, an enzyme essential to microbial sulfate reduction, Wagner et al. (1998) proposed that dissimilatory sulfite reductase was already present in the progenitor of the Bacterial, Archaeal, and Eukaryotic domains or evolved within either *Bacteria* or *Archaea* soon after the split of the domains and was then transferred to the other domain by an early lateral gene transfer event. Isotopic data (Schidlowski et al., 1993; Postgate, 1984; Ohmoto et al., 1993) suggest dissimilatory sulfate reduction (probably correlated to dissimilatory sulfite reductase development) began 2.8 to 3.4 billion years ago. Several types of ancient deposits, including SEDEX and stratiform-copper and Cu-(Pb-Zn) deposits, have been proposed to derive from microbial activity (Trudinger et al., 1972; Haynes, 1986; Ferguson and Skyring, 1995; McGoldrick, 1999). Regardless of the details, it appears that sulfate or sulfite respiration had a very early origin, and this capacity evolved prior to the formation of the major low-temperature, sediment-hosted ore deposits. Results from the Piquette system confirm that this activity in certain low-temperature environments can lead to very high concentrations of potential ore minerals at appreciable rates.

The Piquette biofilms provide possible mineralogical criteria that may be used to examine the role of microorganisms of ancient ores. Although the particle size of ZnS in the Piquette biofilms is extremely small, even at low temperatures, grain growth is inevitable over very long time periods. We interpret time and/or temperature will convert the micron-scale spheres of nanoparticles into micron-sized single crystals, as typify many low-temperature ore deposits. This morphology is similar to the geometry of pyritic framboids, but at a scale several orders of magnitude smaller. The spherical nature of biogenic aggregates may, or may not, be preserved, depending upon the postdepositional history. Notably, micron-scale, spherical PbS particles reported within silicified veins in the Lady Loretta deposit, west Queensland, Australia (Aheimer, 1994), bear an uncanny likeness to the ZnS spheres illustrated in Figure 1 even after 1.6 Ga of burial.

Application of the Piquette geochemical model to ore deposit paragenesis

Ore deposit types: Formation of concentrated ZnS accumulations as the result of sulfate-reducing bacteria activity may have relevance to two general varieties of ore deposits—Mississippi Valley-type and sediment-hosted deposits. Mississippi Valley-type deposits are thought to form within a 50°-to-200°C temperature range (Sverjensky, 1986) and at circumneutral pH (Sicree and Barnes, 1996). Mississippi Valley-type deposits often contain fine-scale, essentially monomineralic bands of microcrystalline sulfides (McLimans et al., 1980; Fowler and L'Heureux, 1996; Sicree and Barnes,

1996) usually associated with organic-rich sections of the host formation (Spirakis, 1985; Gize and Barnes, 1987; Leventhal, 1990). SEDEX deposits also form at relatively low temperatures and are characterized by fine-grained bands of sulfide minerals associated with organic bedding (Eldridge et al., 1993; Large et al., 1998; Painter et al., 1999).

Ore fluid chemistry and evolution: Table 3 lists average stratiform ore fluid chemistry from Haynes (1986) and metal concentrations chosen within the large range of reported values for both deep basinal brines and hydrothermal sea-floor fluids (Sverjensky, 1984; Saunders and Swann, 1990; Doe, 1995). Here, we apply the geochemical approach developed to explain the Piquette mine biofilm mineralization to model the sulfide precipitation and evolution of a fluid appropriate for development of a stratiform ore deposit (Fig. 6; Table 3). Note that a forward modeling approach similar to the one undertaken to understand the generation of the solution in the Piquette mine (Figs. 2 and 3) may be utilized to develop a starting ore fluid for any deposit in question.

Investigation of the resulting thermodynamic model in Figure 6A reveals that sphalerite and galena will coprecipitate first, followed by mackinawite, the low-temperature precursor to pyrite (Benning et al., 2000). The order of sulfide mineral precipitation depends on the specific solution composition chosen to represent the ore fluid, especially on the metal concentrations chosen. Small changes in solution composition (reflecting, for example, changes in pH, carbonate ion concentration, chlorinity, organic ligand availability, and temperature) can affect metal solubility. Metal concentrations can also be a function of the water-rock ratio of the fluid with respect to the source rock. Differences in metal concentrations may delay sphalerite or galena precipitation, potentially yielding

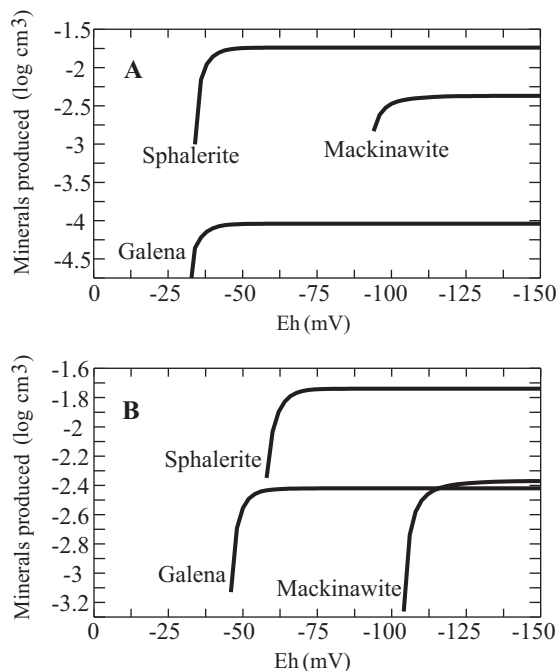


FIG. 6. GWB 3.1 React model of the reduction of stratiform water. Solution compositions are given in Table 3. Pb²⁺ concentrations in each are constrained by calculated solubility with respect to Cerussite in 0.1 and 3 molal Cl⁻ solutions.

single-phase assemblages (Fig. 6A and B). Thus, the model can readily explain both monomineralic and mixed sulfide precipitation.

Prediction of SEDEX deposit paragenesis: Figure 7 presents an example of a paragenetic model designed to simulate ore mineralogy in a hypothetical deposit resulting from two pulses of fluids composed of identical oxidized brines. The progression from left to right in Figure 7 represents the sequence in which metals are removed from the solution as the initially fairly oxidized fluid is reduced. Different rates of sulfate reduction, linked to microbial activity, result in the deposition of different mineral assemblages spatially for each pulse of fluid, which may develop more complex assemblages from many precipitation events in time.

Modeling of ore deposit heterogeneities: The mineralogy of metal sulfide deposits will depend, in part, on whether or not the system is H_2S limited. If H_2S is generated at a rate faster than Zn^{2+} and Pb^{2+} are introduced in the fluid, the redox potential will decrease until FeS precipitation begins. This will result in a deposit less enriched in economically valuable minerals. Alternatively, if H_2S is generated faster than ZnS can precipitate (due to slow ZnS-precipitation kinetics), the fluids may become supersaturated with multiple sulfide minerals. This is unlikely, given the fast precipitation kinetics of metal sulfides (Spirakis, 1985). Thus, so long as Zn^{2+} and Pb^{2+} are introduced faster than sulfide is generated, galena and/or sphalerite precipitation should occur to the exclusion of FeS precipitation. Based on thermochemical sulfate reduction kinetics, a low-temperature sulfide deposit could be formed by abiotic processes within reasonable time frames if the H_2S is generated at higher temperatures and diffuses upwards to a cooler aqueous environment. However, that condition

would create an environment in which the saturation indices of most (or all) potential sulfide minerals are very high and sequential precipitation would not occur.

It is probable that significant heterogeneity exists in both the hydrodynamics of fluid flow through sediments and in the activity of sulfate-reducing bacteria (due to differences in microbial species type, organic substrate supply, and other factors that impact growth rates). These factors may contribute to significant spatial heterogeneity in mineralization. Sulfide bands in the McArthur River HYC (Here's Your Chance) deposit in Northern Australia (one of the largest known Zn-Pb-Ag deposits) were initially classified as monomineralic (Lambert, 1976), but have subsequently been described as a more complex material (Eldridge et al., 1993). Though they are still dominated by sphalerite and galena (Large et al., 1998), Eldridge (1993) noted that truly monomineralic character only occurs in patches that extend for no more than a few tens of microns in any direction. Thus, the spatial variability in the mineralogy of the HYC deposit can be modeled as the result of variations in biological activity and solution chemistry consistent with the generalized model presented in Figure 7.

Conclusions

The thermodynamic model developed to explain in situ deposition of ZnS within natural biofilms over less than 30 yr has been applied to a range of possible ground-water and ore-forming solutions. Fluids introducing zinc must be flowing at a sufficient rate to ensure that sulfide produced by sulfate-reducing bacteria can be effectively titrated out by ZnS (or PbS) precipitation. Notably, the Zn concentration does not need to be high. In fact, a few tens of ppb Zn, as occur in many ground-water solutions, are sufficient so long as the fluid flow rate is appropriate.

The results of the modeling contribute a general understanding of how separation of different sulfides occurs as the result of individual mineral-phase solubility and the kinetics of sulfate reduction. The approach may represent a useful tool in interpreting complicated paragenetic sequences such as encountered in the HYC deposit. Application of the model coupled with interpretation of mineralogical fabrics can constrain geochemical conditions such as aqueous sulfide concentration (if high, more than 1 sulfide mineral would likely precipitate) and temperature (affects twinning and structural overgrowths). These conditions are important factors in determining the role of thermochemical and/or biological sulfate reduction in ore deposits.

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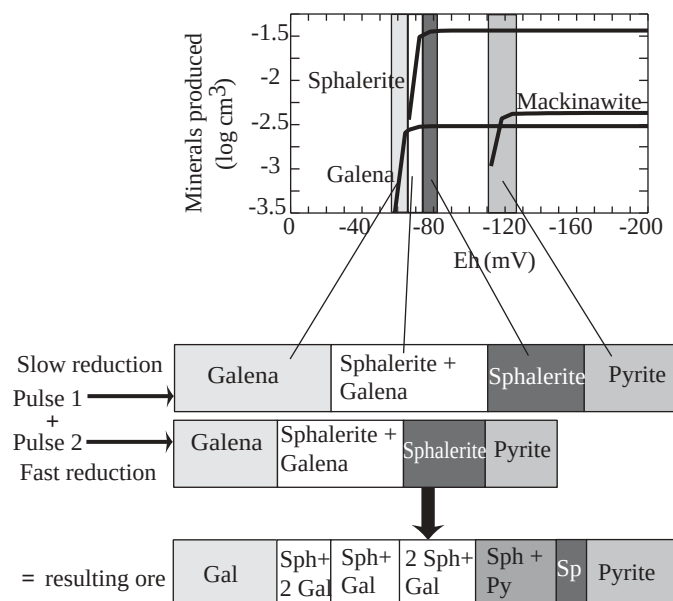


FIG. 7. Modeled paragenesis of hypothetical SEDEX deposit. Different rates of sulfate reduction, linked to microbial activity, result in the deposition of different mineral assemblages spatially for each pulse of fluid. The sum of two chemically identical pulses results in the lowest assemblage. Large et al. (1998) propose ~10,000 pulses formed each of the eight ore lenses in the HYC deposit, one of the largest Pb-Zn-Cu SEDEX deposits in the world.

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