

Microbial contributions to cave formation: New insights into sulfuric acid speleogenesis

Annette Summers Engel*
Libby A. Stern
Philip C. Bennett

Department of Geological Sciences, Research Group for Microbial Geochemistry, University of Texas, Austin, Texas 78712, USA

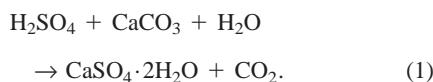
ABSTRACT

The sulfuric acid speleogenesis (SAS) model was introduced in the early 1970s from observations of Lower Kane Cave, Wyoming, and was proposed as a cave-enlargement process due to primarily H_2S autoxidation to sulfuric acid and subaerial replacement of carbonate by gypsum. Here we present a reexamination of the SAS type locality in which we make use of uniquely applied geochemical and microbiological methods. Little H_2S escapes to the cave atmosphere, or is lost by abiotic autoxidation, and instead the primary H_2S loss mechanism is by subaqueous sulfur-oxidizing bacterial communities that consume H_2S . Filamentous “*Epsilonproteobacteria*” and *Gammaproteobacteria*, characterized by fluorescence in situ hybridization, colonize carbonate surfaces and generate sulfuric acid as a metabolic byproduct. The bacteria focus carbonate dissolution by locally depressing pH, compared to bulk cave waters near equilibrium or slightly supersaturated with calcite. These findings show that SAS occurs in subaqueous environments and potentially at much greater phreatic depths in carbonate aquifers, thereby offering new insights into the microbial roles in subsurface karstification.

Keywords: cave, hydrogen sulfide, geomicrobiology, sulfur-oxidizing bacteria, fluorescence in situ hybridization, carbonate dissolution.

INTRODUCTION

Karst landscapes form where soluble rocks dissolve, resulting in caves and conduit drainage systems that are important water, hydrocarbon, and tourism resources (e.g., White, 1988; Ford and Williams, 1989; Palmer, 1991). The classic model for karst development (speleogenesis) is carbonic acid dissolution of carbonate rocks, usually at shallow depths rarely below the water table. More recently, sulfuric acid speleogenesis (SAS) was proposed by S.J. Egemeier from work in Lower Kane Cave, Wyoming (Egemeier, 1981; Hill, 1990; Jagnow et al., 2000). On the basis of observations of H_2S -bearing thermal springs, extensive gypsum deposits, and gypsum-replaced limestone cave walls in Lower Kane Cave, Egemeier (1981) proposed the original SAS model to include the volatilization of H_2S from the groundwater to the cave atmosphere and H_2S oxidation to sulfuric acid on moist subaerial cave-wall surfaces, where the acid reacts with and replaces the carbonate host rock with gypsum. Gypsum easily dissolves into groundwater, and the net result is the removal of mass and an increase in void volume.



SAS is now recognized in several active sulfidic caves in the United States, Romania, It-

aly, and Mexico (Hubbard et al., 1990; Galdenzi and Menichetti, 1995; Sarbu et al., 1996; Hose et al., 2000), as well as in large ancient hypogene caves, e.g., Carlsbad Cavern, New Mexico (Hill, 1990; Polyak et al., 1998). In addition to subaerial processes, SAS in the Guadalupe Mountains has also been attributed to sulfuric acid dissolution at or just below the water table (Hill, 1990; Palmer, 1991; Jagnow et al., 2000).

H_2S is a rich energy source for microorganisms, and the springs and discharge streams in Lower Kane Cave are colonized by thick, filamentous microbial mats. However, the role of sulfuric acid-generating microorganisms in cave formation, although previously alluded

to, has never been substantiated (Hubbard et al., 1990; Angert et al., 1998; Hose et al., 2000; Vlasceanu et al., 2000; Engel et al., 2001). We report here a reexamination of the basic SAS process in Lower Kane Cave, taking into consideration the presence of sulfur-oxidizing bacteria in subaqueous microbial mats. We found that, in contrast to the classic SAS model, only a small percentage of the dissolved H_2S volatilizes into the cave air; instead, most of the H_2S is consumed within the subaqueous environment by microbial oxidation.

LOWER KANE CAVE, WYOMING

Lower Kane Cave is forming in Madison Limestone within the Little Sheep Mountain anticline, Bighorn Basin, on the western margin of the Bighorn and Pryor mountain belts. We describe here results from only 1 of 4 springs (Upper Spring) that discharge into the cave passage and its 17-m-long microbial mat-filled outflow channel (Fig. 1); similar results were found at the other spring-stream complexes. Water and gas compositions were characterized seasonally for three years by standard and specially developed methods¹ for the cave environment. At the study spring, anaerobic sulfidic water discharges ($\sim 21.6 \text{ m}^3 \cdot \text{h}^{-1}$)

¹GSA Data Repository item 2004061, supplemental methods and Table DR-1, is available online at www.geosociety.org/pubs/ft2004.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301-9140, USA.

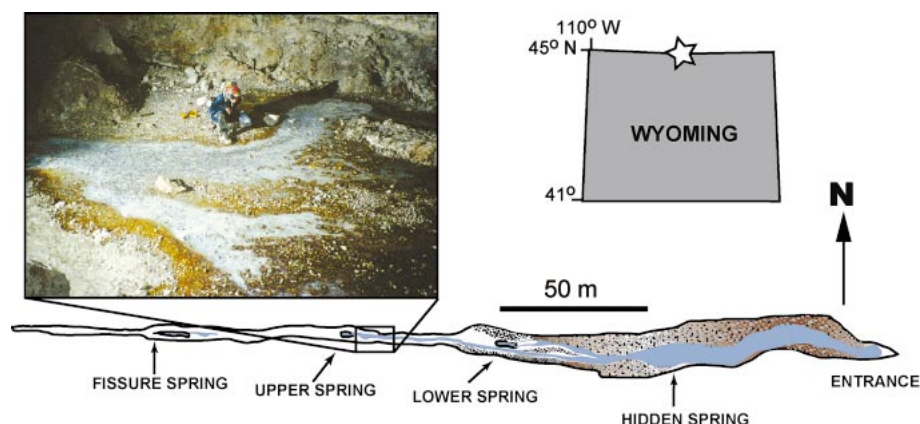


Figure 1. Site location map and plan-view cave map showing main spring sites and stream channel in Lower Kane Cave, Wyoming (modified from Egemeier, 1981). Photograph shows white filamentous microbial mats downstream from Upper Spring orifice (flow is left to right).

*E-mail: aengel@mail.utexas.edu.

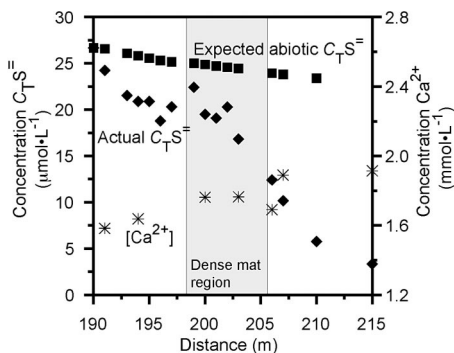


Figure 2. Concentration profiles vs. depth. Diamonds—actual $C_T S^=$ values measured in cave stream. Asterisks—dissolved $[Ca^{2+}]$. Squares—total dissolved sulfide ($C_T S^=$) following abiotic (autoxidation and volatilization) loss.

into a 20 m², 0.5-m-deep pool (Fig. 1). A representative water composition at 21.5 °C and pH 7.36 (40% H₂S:60% HS⁻; pK = 7.04) is as follows: Ca²⁺, 1.58 mmol·L⁻¹; SO₄²⁻, 1.13 mmol·L⁻¹; Cl⁻, 0.12 mmol·L⁻¹; HCO₃⁻, 3.39 mmol·L⁻¹; and total concentration of dissolved sulfide ($C_T S^=$), 34 μmol·L⁻¹ (Fig. 2). There is no detectable dissolved O₂ (Table DR-1; see footnote 1). Along the proximal reach of the outflow channel, $C_T S^=$ decreases slightly as the water undergoes a transition from anaerobic to dysaerobic (O₂ = 0.1–5.0 μmol·L⁻¹) (Fig. 2), accompanied by a visible change in microbial mat morphology. Downstream, corresponding to an increase in dissolved O₂, a 2–5-cm-thick, geochemically stratified microbial mat develops. The mats abruptly terminate where dissolved O₂ exceeds 45 μmol·L⁻¹, and here $C_T S^=$ rapidly decreases to <6 μmol·L⁻¹ (Fig. 2; Table DR-1 [see footnote 1]).

HYDROGEN SULFIDE TRANSPORT AND REACTION

On the basis of Egemeier's (1981) original SAS model that subaerial replacement of limestone by gypsum was a significant mechanism for cave enlargement, we initially hypothesized that most of the dissolved sulfide would quickly escape into the cave atmosphere as H₂S(g). We tested this hypothesis by measuring the concentrations of dissolved and atmospheric gases (permanent, hydrocarbon, and sulfur gases) and by directly determining the actual flux of H₂S(g) from the cave water to the atmosphere (see footnote 1). More than 20 real-time flux measurements were made at ~2 m intervals from the Upper Spring orifice to the end of the microbial mats (Figs. 1 and 2), and 400 gas samples were taken throughout the cave. Because of the fugitive nature of some sulfur gases, measurements were made immediately in the cave by direct-inject field-based gas chromatography (GC).

Stream velocity and discharge were determined by dilution tracing and varied from 0.5

m·s⁻¹ along the 5 m channel proximal to the Upper Spring to 0.2 m·s⁻¹ at the mat's downstream terminus. At the Upper Spring, the influx of dissolved sulfide is ~8700 μmol·min⁻¹; the concentration of H₂S(g) at the air-water interface averages 30 ppmv (Table DR-1; see footnote 1), corresponding to an average H₂S(g) flux of 44 μmol·m⁻²·min⁻¹. Over the entire length of the Upper Spring microbial mats, however, volatilization of H₂S accounts for <8% of the total influx of $C_T S^=$, suggesting that the bulk of the $C_T S^=$ is lost by other mechanisms in the subaqueous environment, not by volatilization. The measured H₂S(g) flux was compared to a theoretical volatilization rate calculated by using the two-film model of Liss and Slater (1974) (see footnote 1), which yields an estimated flux of 23 μmol·m⁻²·min⁻¹, with a 13 min first-order half-life. This value compares very closely to the actual half-life of ~6 min based on the mean measured H₂S(g) flux.

The actual decrease in $C_T S^=$ in the cave stream is much faster than could be accounted for by volatilization alone (Fig. 2). Another possible mechanism for $C_T S^=$ loss is abiotic autoxidation, which was evaluated by using published kinetic data determined at 25 °C for dilute waters (Millero et al., 1987):

$$\frac{d[H_2S]_T}{dt} = k[H_2S]_T[O_2], \quad (2)$$

where k is the second-order oxidation-rate constant and t is time. At pH values near the pK of H₂S, k consists of two components, k_0 and k_1 , corresponding to the oxidation of H₂S and HS⁻, 11 and 48 L·mol⁻¹·h⁻¹ respectively (Millero et al., 1987). $C_T S^=$ autoxidation is therefore extremely slow in the dysaerobic, pH 7.3 stream water at 20 μmol·L⁻¹ $C_T S^=$ and a constant O₂ of 20 μmol·L⁻¹ (conditions at the 201 m location in the cave, downstream from the Upper Spring; Fig. 2; Table DR-1 [see footnote 1]). The pseudo first-order autoxidation half-life is determined to be 866 h if a second-order rate constant of 40 L·mol⁻¹·h⁻¹ is used (see footnote 1; $t_{1/2} = (\ln 2)/k[O_2]$) (Millero et al., 1987). From the sum of the actual volatilization and the theoretical autoxidation along the 17 m stream-mat complex, if an average flow velocity of 0.3 m·s⁻¹ at ambient conditions is assumed (Fig. 2), the total potential abiotic loss of $C_T S^=$ would result in a very slow first-order loss of sulfide and significant $C_T S^=$ remaining at the cave exit.

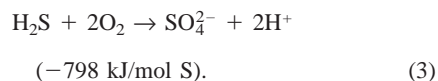
The observed sulfide loss is still much faster than can be accounted for from the combined effects of abiotic volatilization and autoxidation (Fig. 2). From the Upper Spring stream-channel outflow, $C_T S^=$ decreases moderately, from 24 to 20 μmol·L⁻¹ over 5 m (~0.2 min) (Fig. 2). Over the middle 7 m reach, $C_T S^=$ remains approximately constant at 20 μmol·L⁻¹

until near the end of the microbial mats. Over the distal 5 m reach, $C_T S^=$ decreases abruptly to <10 μmol·L⁻¹ at the mat terminus, and sulfide is undetectable 10 m downstream (Table DR-1; see footnote 1).

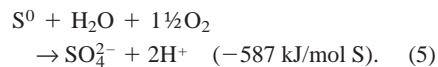
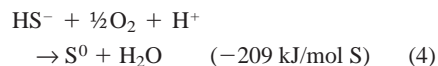
The distinctly steep and concave-down concentration versus distance profile (Fig. 2) is unlike the expected shallow, concave-up first-order loss profile characteristic for the abiotic loss mechanisms and requires microbially catalyzed sulfide consumption. The relatively constant $C_T S^=$ in the middle stream reach further suggests that sulfide loss is offset in part by in situ biotic H₂S production by sulfate-reducing bacteria, and we have cultured a variety of sulfate reducers from the mats (Engel et al., 2002). Therefore, the extremely rapid decrease in $C_T S^=$ near the microbial mat terminus suggests subaqueous biotic consumption of both allochthonous and autochthonous sulfide. The high background [SO₄²⁻] in the stream water, however, makes it impossible to measure small changes in oxidation byproducts.

SUBAQUEOUS MICROBIAL DISSOLUTION OF CARBONATES

We observed deeply corroded native carbonate fragments (pebble- to cobble-size limestone clasts) in the cave stream that showed dissolution effects only on surfaces exposed to stream water and the filamentous microbial mats. The dominant mechanism for $C_T S^=$ loss is by subaqueous microbial oxidation, and most sulfur-oxidizing bacteria oxidize H₂S completely to sulfate with a substantial energy yield:



Others initially form elemental sulfur (S⁰) as an intermediate that is stored intracellularly and further oxidized during periods of limiting sulfide:



The net result is the local production of sulfuric acid that directly attacks the geologic matrix supporting the microbial community. Although some sulfur-oxidizing bacteria are acidophiles (Johnson, 1998), most are neutrophilic, and colonization of carbonate surfaces therefore buffers the excess acidity and maintains pH homeostasis (Engel et al., 2001).

We previously characterized the microbial mat community by using culture-independent molecular methods, including 16S rRNA gene retrieval and fluorescence in situ hybridization

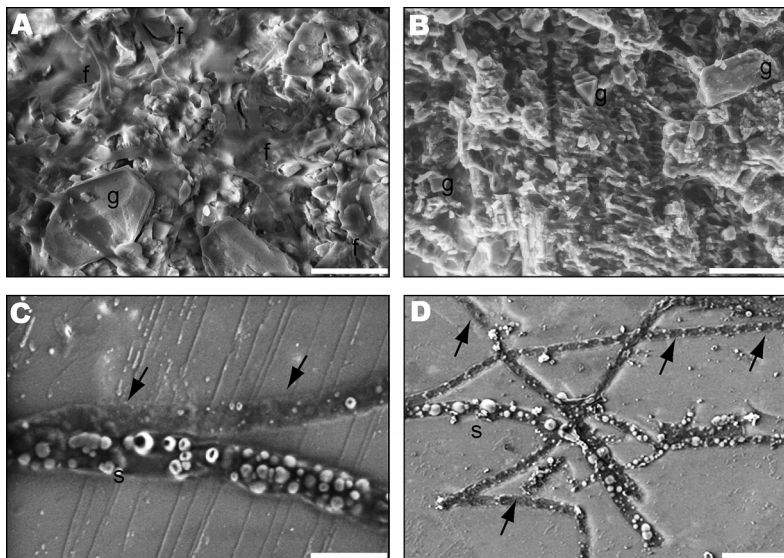


Figure 3. Environmental scanning electron microscope micrographs showing surface textures of native limestone (images A and B) and calcite from field chambers (images C and D). **A:** Biofilm and filaments (f) coating surface and gypsum (g) crystals on native limestone (0.9 torr, 12 kV). Scale bar represents 10 μm . **B:** Biofilm showing deeply etched limestone surface filled with gypsum (6.4 torr, 20 kV). Scale bar represents 50 μm . **C:** Calcite chip surface showing two filament morphologies, one with intracellular sulfur (s) globules and another filament without (black arrows) (1.0 torr, 6.0 kV). Scale bar represents 5 μm . **D:** Calcite chip surface with two filament morphologies showing deep trenches directly under filaments without intracellular sulfur (black arrows) in contrast to shallow trenches under filaments with sulfur (1.6 torr, 4.0 kV). Scale bar represents 20 μm .

(FISH) with 16S rRNA-specific probes targeting mat bacterial groups (Amann et al., 1995; Engel et al., 2003). The dominant microorganisms in Lower Kane Cave mats are phylogenetically grouped within the “*Epsilonproteobacteria*.” Less abundant communities of known sulfur-oxidizing bacteria from the genera *Thiothrix* (*Gammaproteobacteria*) and *Thiobacillus* (*Betaproteobacteria*) are also present (Engel et al., 2003). Although most “*Epsilonproteobacteria*,” including those identified from Lower Kane Cave, have not been obtained in pure cultures, many of the successfully cultured epsilonproteobacterial groups oxidize reduced sulfur compounds by

using molecular oxygen (under microaerophilic conditions) or alternative electron acceptors, such as nitrate or metals (e.g., Takai et al., 2003). We cautiously hypothesize that the Lower Kane Cave organisms are also sulfur oxidizers on the basis of phylogenetic affiliations and habitat geochemistry. Most cultured “*Epsilonproteobacteria*” do not store sulfur intracellularly, in contrast to members of the *Gammaproteobacteria*, specifically *Thiothrix* spp. (Larkin, 1989).

Examination of the native carbonate surfaces by environmental scanning electron microscope (ESEM) (see footnote 1) reveals a complex reaction environment of dissolving

calcite, microbial filaments, and secondary gypsum (Figs. 3A and 3B). The stream chemistry, however, is at near equilibrium to slightly supersaturated with respect to calcite and undersaturated with respect to gypsum (Table DR-1; see footnote 1). The observed carbonate dissolution and gypsum precipitation associated with a surface biofilm suggest that SAS occurs within a mineral surface microenvironment maintained by the microbial community, and not by changes in bulk aqueous geochemistry.

To distinguish subaqueous microbial SAS from bulk geochemical controls, we used field chambers and a modification of the buried-slide technique (Parkinson et al., 1971). Sterile and nonsterile field chambers (covered with 0.1 μm filters and 0.5 mm mesh, respectively) and mesh-enclosed slides containing chips of Iceland spar calcite and smoothly polished native limestone fragments were submerged in the stream and mats (see footnote 1). The water exiting the Upper Spring pool has a calculated equilibrium partial pressure of CO_2 ($p\text{CO}_2$) of $10^{-2.1}$ atm and is in approximate equilibrium with calcite [saturation index of calcite (SI_{cal}) ≈ -0.06], but significantly undersaturated with gypsum (SI_{gyp} ≈ -1.6). However, although the waters are in equilibrium or supersaturated with calcite, ESEM examination of the chamber calcite revealed etching localized where microbial filaments or biofilms are attached to the mineral surfaces (Figs. 3C and 3D), whereas sterile calcite chips exposed to the stream water show no microbial colonization and no apparent dissolution. Two filament types were observed on nonsterile calcite chips, one containing sulfur globules and another without (Fig. 3C). Dissolution trenches deeper than trenches associated with sulfur-containing filaments (Fig. 3D). Differences in dissolution intensity at the microbial filament may correspond to the predominance of each of the two sulfide oxidation mechanisms (equation 3 versus equation 4); greater localized acidity may be generated under the filaments that do not store sulfur (equation 3).

The microbial mats consist of predominantly filamentous “*Epsilonproteobacteria*” with less abundant *Gammaproteobacteria* (*Thiothrix*) (Engel et al., 2003) (Fig. 4A), and we applied FISH probes to the experimental chip surfaces, including probe LKC1006 targeting a filamentous epsilonproteobacterial group dominant by biovolume (Engel et al., 2003), probe GAM42a for *Gammaproteobacteria*, and a general bacterial probe set EUB338I-III mix (see footnote 1). By using confocal laser scanning microscopy, all three probes on rock and mineral surfaces showed positive hybridization signals (Figs. 4B and 4C). Exceptionally bright hybridization signals for each of the probes indicated high rRNA content, sug-

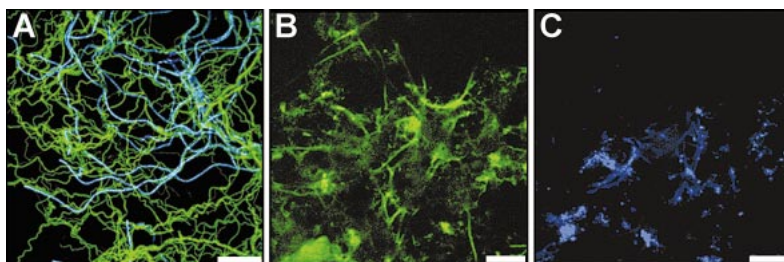


Figure 4. **A:** Fluorescence in situ hybridization (FISH) of microbial mat sample from mat terminus with probes EUB338I-III mix (green) and GAM42a (light blue), specific for all Eubacteria (dominated by filamentous “*Epsilonproteobacteria*”) and *Gammaproteobacteria* (which includes *Thiothrix* spp.), respectively. **B and C:** FISH of filaments attached to polished chip of native limestone from mesh-covered buried slides using probe LKC1006 (**B:** green) specific for dominant epsilonproteobacterial group in Lower Kane Cave microbial mats and with probe GAM42a (**C:** light blue). Scale bar in all images represents 20 μm .

gesting metabolically active populations when the samples were retrieved (Amann et al., 1995). Nearly all observed filaments simultaneously hybridized with the EUB338I-III mix and LKC1006 probes on limestone surfaces (Fig. 4B), and fewer filaments hybridized with the GAM42a and EUB338I-III mix probes (Fig. 4C). The FISH results genetically identify the two filamentous sulfur-oxidizing bacterial groups that colonize subaqueous carbonate surfaces, one that stores sulfur and one that does not, consistent with the ESEM observations.

MICROBIAL SULFURIC ACID SPELEOGENESIS

The rapid loss of sulfide from the stream (Fig. 2), the carbonate dissolution associated with microbial filaments (Figs. 3C and 3D), and the dominance of “*Epsilonproteobacteria*” on the experimental limestone surfaces (Figs. 4B and 4C) support a hypothesis that these organisms oxidize H₂S to sulfuric acid and suggest a direct microbial role in SAS. Previously, part of the SAS model relied on H₂S volatilization from the cave stream to the cave atmosphere (Egemeier, 1981; Palmer, 1991), yet we find negligible volatilization or abiotic autoxidation of C₇S⁼ in the cave stream. Instead, C₇S⁼ is consumed by subaqueous sulfide-oxidizing bacteria. This finding suggests that cave enlargement via dissolution of the cave floor is microbially mediated. In the cave stream, the system is very close to equilibrium, and the calcite dissolution rate is a function of [Ca²⁺], pCO₂, and solution pH. The rate has a nonlinear dependence on the degree of undersaturation (Ω) that can be described as the difference between the actual and equilibrium saturation pH ($\Delta\text{pH} = 0.5 \log \Omega$) (e.g., Berner and Morse, 1974). At conditions of low Ω ($\Delta\text{pH} < 0.15$), such as found in the bulk stream water receiving diffuse proton input from abiotic sulfide oxidation, calcite dissolution is very slow with little effect from small changes in pH. In contrast, where bacterial filaments are in contact with carbonate, excess acidity is focused at the reacting surface (equation 3) and local Ω increases. Although the stream water is near calcite equilibrium, the [Ca²⁺] increases along the stream region where microbial growth is greatest (Fig. 2), whereas [SO₄²⁻] increases only slightly (Table DR-1; see footnote 1). Our observations show that sulfur-oxidizing bacteria colonize subaqueous carbonate surfaces, localize dissolution by generating acidity, and therefore are integral to sulfuric acid speleogenesis.

IMPLICATIONS

While a microbial role to cave formation has been suggested (Hubbard et al., 1990; Angert et al., 1998; Hose et al., 2000; Vlasceanu et

al., 2000; Engel et al., 2001), previous explanations for SAS assumed abiotic chemical and hydrologic controls that predominantly operate either near a shallow groundwater table, because of oxygen requirements for abiotic processes, or subaerially after H₂S volatilization, on the basis of extensive gypsum in these cave systems (Egemeier, 1981; Hill, 1990; Palmer, 1991; Galdenzi and Menichetti, 1995). Our work in Lower Kane Cave confirms that some H₂S volatilizes into the cave atmosphere and consequently subaerial speleogenesis occurs; however, its long-term rate is unknown. The small H₂S volatilization flux today cannot explain present SAS processes. Instead, we find that almost all of the H₂S is consumed by subaqueous sulfur-oxidizing bacteria. The bacteria drive SAS by attachment to carbonate surfaces and generation of sulfuric acid, which focuses local carbonate undersaturation and dissolution. Furthermore, the sulfur-oxidizing bacteria we identified are metabolically active under low oxygen tension and catalyze sulfide oxidation where autoxidation would be kinetically limited. Therefore, microbial catalysis extends the phreatic depths to which porosity and conduit enlargement could occur in carbonate systems, including oil-field reservoirs and aquifers. The metabolic consequences of an active microbial ecosystem change the model for sulfuric acid speleogenesis.

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