Microbiology and Ecology of Filamentous Sulfur Formation

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A highly motile chemoautotrophic strain of hydrogen sulfide–oxidizing bacteria from coastal seawater produces solid sulfur filaments of dimensions 0.5 to 2.0 micrometers by 20 to 500 micrometers. Filamentous sulfur is rapidly produced by direct excretion by a vibrioid organism, and the newly produced filaments are thickened by the deposition of sulfur by other members of the population. Microscopic observations of the flocculent discharge material collected from diffuse-flow hydrothermal vents (9°N, East Pacific Rise) revealed that the material from this source is composed largely of filamentous sulfur of morphology nearly identical to that obtained in the model laboratory system.

Sulfur oxidation in the marine environment by free-living microbes typically occurs at interfaces where oxygen and H_2S coexist, such as between anaerobic marine sediments and the aerobic water column (*1*), in poorly circulated marine water bodies (*2*), and at submarine hydrothermal vents (*3*). To further understand the microbiology of hydrothermal vents, we established enriched environments for sulfur-oxidizing bacteria in stirred reactors where a steady stream of oxygenated seawater (dilution rate, ≈ 1 hour^{-1}) was enriched with H_2S (steady-state concentration, 1.0 to 1.5 mM) by diffusion of the pure gas through the walls of immersed silicone rubber tubing. When coastal seawater that had been overlying anoxic marine sediments was used as an initial inoculum, a self-perpetuating enrichment became established: A mass of white flocculent material reminiscent of fungal mycelial growth repeatedly filled the interior of the reactor within 1 or 2 days of reinoculation (Fig. 1, A through C).

The flocculent material was composed of irregular filaments 0.5 to 2.0 μm wide and from 20 to 500 μm in length (Fig. 1D). The filaments would not take up the nuclear stain acridine orange (*4*). Organisms that stained were instead vibrios (dimensions when observed by phase microscopy, 0.4 to 0.6 μm by 2.5 to 3.0 μm) that were entrained in high numbers within the flocculent clumps of filaments (compare Fig. 1, E and F).

Elemental analysis of the produced reactor material revealed that it was 82% sulfur by weight and 18% combustible organic matter. Analysis by energy dispersive spectroscopy (EDS) showed that the filaments were composed entirely of the element sulfur (elements of atomic weight less than fluorine and greater than cadmium are not detectable by EDS) (*5*).

The white flocculent material could be completely wetted by water, suggesting that the filaments may possess a hydrophilic coating functionally analogous to the amphiphilic layers found on sulfur globules formed biogenically (*6*) or chemically (*7*) or may be coated by a hydrophilic organic material. The filaments do not possess the micellular liquid-like properties of intra- or extracellular hydrophilic sulfur globules. They are rigid, birefringent under polarized light, and therefore probably crystalline. Filamentous sulfur formation requires sulfide concentrations higher than 400 μM (optimum, 1.5 mM) and the presence of microorganisms (*8*).

When H_2S-enriched seawater and small tufts of filamentous sulfur from a reactor were drawn into a 0.2 mm by 4 mm flat capillary tube, counter-opposing H_2S-oxygen gradients became established, resulting in the outgrowth of sulfur filaments (Fig. 2A). The interior of the newly formed outgrowth was relatively open, consisting of long (300 μm), irregular filaments of sulfur radiating outward from the central holdfast. Vibrioid organisms were attached radially along, in this case, the outer 30 to 50 μm of the sulfur filaments (white arrow and inset). In the interior and probably anoxic regions of the mycelial structure, filaments were essentially free of microorganisms, suggesting that the microorganisms remained attached to the filaments only in the zone where oxygen and H_2S coexisted.

The following sequence of events led to filamentous sulfur formation within the capillaries. Within approximately 10 min of setup of the capillary, a swarm of highly motile vibrio-shaped microorganisms aggregated into a 100-μm band at the H_2S-oxygen interface. Many of the organisms reversibly attached end-on to the inner surface of the glass capillary. Some remained attached long enough to deposit a small droplet of sulfur on the glass surface. In 20 to 40 min, the sulfur droplets carpeted the glass surface in the region of the interface, and a few bacteria-covered filaments began to emerge from the glass surface. In time, a filament several hundred micrometers in length was formed that was completely covered by radially attached microorganisms (Fig. 2B). When the oxygen-H_2S interface began to migrate away from a given region (as can be judged by the migration of the remaining bacterial swarm), the attached microorganisms abandoned the site until only the naked sulfur filaments remained (Fig. 2, B through D). It was not until most of the population was attached to the various surfaces in the preparation that initial filament formation was directly observable. The sulfur filaments were formed by direct excretion by a single organism (Fig. 3). The newly formed filaments (Fig. 3B) were substantially smaller in diameter than mature filaments (Figs. 1D and 2D) and grew longer at rates of >3 μm min^{-1} (Fig. 3B, dashed white arrows). Swarming organisms readily attached to the new filaments (Fig. 3B, black arrow) and presumably deposited droplets of sulfur onto the filament. If a large number of organisms became attached to the filament, as in Fig. 2B, it was thickened substantially by the continued

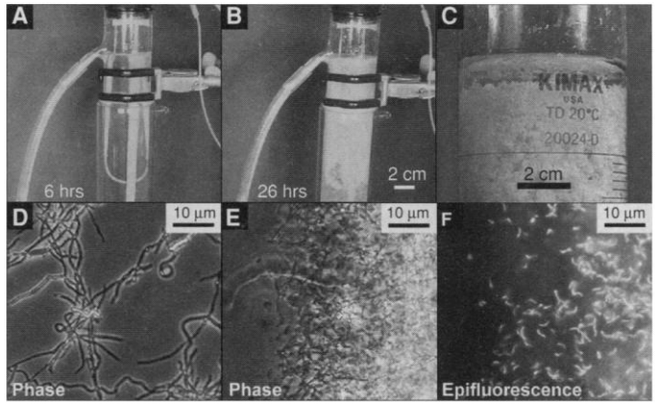

Fig. 1. Macroscopic and microscopic appearance of the flocculent material present in a continuous flow, H_2S-enriched seawater reactor. (**A** and **B**) Production of flocculent material in the reactor 6 and 26 hours after inoculation. Hydrogen sulfide was diffused (by way of a semicircular loop of silicone tubing) into stirred, oxygenated seawater (supplied by the large vertical tube). Dilution rate of reactor, 1 hour^{-1}; nominal sulfide concentration, 1.2 mM. (**C**) Appearance of the material when removed from reactor to a graduated cylinder after 26 hours. (**D**) Phase microscopic appearance of flocculent material from reactor. Irregular filaments are nominally 0.5 to 2 μm in width and range from 20 to 500 μm in length. (**E**) Phase and (**F**) acridine orange epifluorescence images of the same field of a filamentous clump of reactor material.

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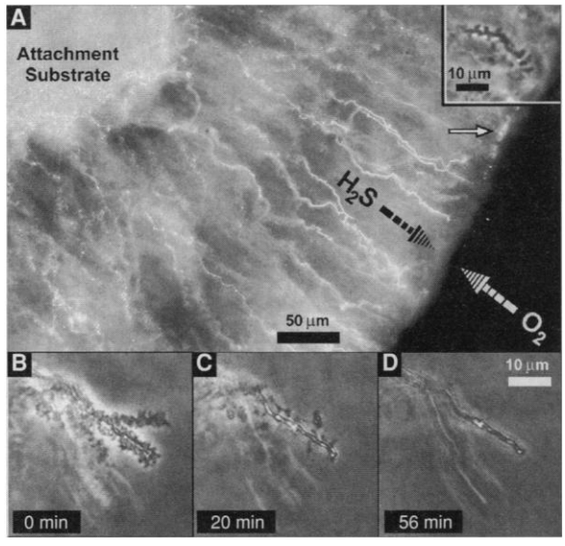

Fig. 2. Microscopic visualization of sulfur filaments and sulfur-producing microorganisms. (**A**) Sulfur filaments (irregular white lines) radiating from a holdfast of compacted sulfur filaments present in a 0.4 mm by 4.0 mm capillary tube. The filaments visible are those that lie within the microscopic plane of focus. The background "glow" is light scattered by filaments above and below the plane of focus. The small white arrow indicates one locus of microorganisms. (**Inset**) Higher magnification view of vibrio-shaped microorganisms attached to sulfur filament. (**B** through **D**) Organisms detaching from sulfur filament when environmental conditions become sub-optimal. Shown is the time sequence of organisms leaving a filament when the H_2S-oxygen interface moved to the right.

deposition of sulfur. The sulfur solidified rapidly; filaments (Fig. 3) become essentially rigid at the point of excretion from the cells.

A possible organelle associated with sulfur excretion appears to be a discrete multilaminar membrane structure located at one end of the cell (Fig. 4, white arrows). The membrane structures were 27 to 33 nm thick, composed of 4 to 5 lamellae that were approximately 75 Å thick (Fig. 4B), and may be associated with the interior of the cell membrane (Fig. 4C). The structures have been observed in a number of configurations within the cell (Fig. 4) and may reflect either different sections of a single U-shaped membranous structure or multiple patches of membrane within the cell.

Filamentous sulfur formation may be a widespread phenomenon. It forms in flowing seawater enrichments intended for *Thiovulum* spp. (9) and in tidal salt marsh creeks, where filament mats overlay small, submerged animal burrows, which transport sulfide from the deeper reaches of the organic-rich sediments to the surface (*10*). Filamentous sulfur may be an abundant product of nonsymbiotic microbial sulfide oxidation below the surface of warm-water submarine hydrothermal vents. Recent descriptions of an extensive discharge from so-called "snowblower" vents indicated that a large bacterial bloom occurred during and immediately after an eruption at the East Pacific Rise at 9°45′ to 9°52′N (*11*, *12*). Massive amounts of a white flocculent or filamentous mat-like material were released and accumulated into 5-cm-thick mats (*11*, *13*). Discharge material from a 9°N snowblower vent (*14*) and samples collected from warm-water vents at Atlantic and Pacific vent sites were visually similar to the filamentous sulfur material produced by the vibrio in our laboratory reactors, both when visualized in intact flocs (Fig. 5, A and B) and when sheared apart by vigorous agitation (Fig. 5, C and D). The vent material was white and hydrophilic in nature, had a high sulfur content (*13*), and possessed other identical properties (*15*).

It was postulated that snowblower-vent filaments were microorganisms, possibly sulfur-oxidizing archaeal organisms (*11*). Given our present observations, it is likely that the filaments may instead be sulfur and the mechanism of their formation may be comparable to that described in our model system.

Filamentous sulfur formation seems to occur in sulfidic environments characterized by active fluid movement. These microorganisms are retained within the environment by the deposition of sulfur as en-

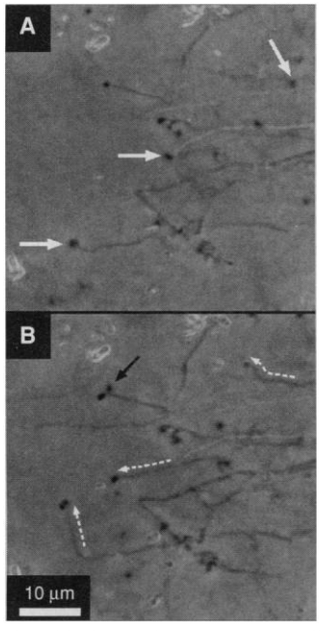

Fig. 3. Individual organisms excreting sulfur filaments. (**A**) Organisms that were actively excreting filamentous sulfur, indicated by white solid arrows. (**B**) Same field 3 min later. The length of the filaments excreted in the 3 min is delineated by the dashed white arrows. The solid black arrow indicates an organism that swam in and attached to an existing filament.

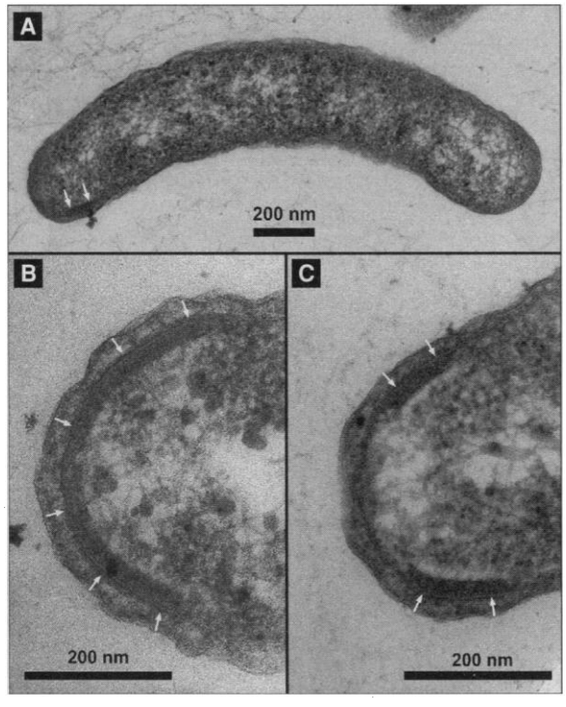

Fig. 4. Thin-section transmission electron microscopic visualization of probable sulfur excretion organelle. (**A**) Longitudinal section of sulfur-filament–producing organism. (**B** and **C**) Enlarged view of multilaminar polar membrane structures. Membrane structures are indicated by white arrows. The sample was prepared according to (*17*).

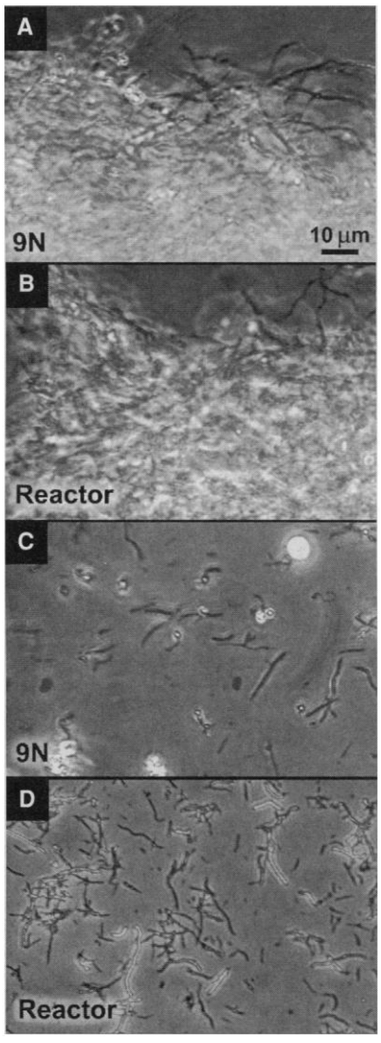

Fig. 5. Phase microscopic comparison of flocculent material from the 9°N snowblower vent and the laboratory reactor. (**A** and **B**) Intact flocs. (**C** and **D**) Filamentous material dispersed by vigorous physical mixing. Symbols: 9N, samples obtained from 9°N snowblower vent; Reactor, samples obtained from the laboratory reactor.

tangling, long, irregular filaments rather than as globular or amorphous sulfur, as found with most known sulfide oxidizers. By modification of the turbulent boundary layer, mats of these filaments may also be important in regulating the local microenvironment (for example, O_2-H_2S gradients) in which the organisms reside.

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5. Filaments also completely dissolved under the microscope when seawater was gradually exchanged with ethanol, and they melted into spheres when illuminated by high-intensity near-ultraviolet (UV) light at the absorption band for sulfur (excitation bandpass, 340 to 390 nm). Both filaments and finely ground commercial sublimed elemental sulfur absorb sufficient near-UV radiation to be heated to their melting point, even when immersed in water. Filamentous sulfur washed in distilled water and steam sterilized (three times at 110°C) supported chemolithoautotrophic growth of two *Thiomicrospira* species (*16*). Both organisms grew nearly as quickly on the filamentous sulfur as they did on soluble thiosulfate, and twice as fast as when grown on commercial sublimed elemental sulfur. Ethanol solubility and ease of microbial utilization probably reflect the hydrophilic nature of the filaments.
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8. Sterile reactors and inoculated reactors poisoned with glutaraldehyde or sodium azide failed to produce filaments. Radioisotopic experiments using cleaned suspensions of the vibrio (>99.5% vibrios) showed that they were able to fix CO_2 maximally at sulfide concentrations between 500 and 1500 μM and continued fixing CO_2 at 2000 μM, a concentration that is toxic for previously described hydrothermal-vent sulfur oxidizers (*16*).
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18. We thank S. J. Molyneaux, D. Wiebe, and L. Hare for assistance with technical aspects of this work. Woods Hole Oceanographic Institution contribution 9432. Supported by grant IBN96-30054 from NSF.

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The Isotopic Oxygen Nightglow as Viewed from Mauna Kea

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Optical spectra of the terrestrial nightglow in the 520- to 900-nanometer region, as measured by the W. M. Keck telescope on Mauna Kea in Hawaii and the associated high-resolution echelle spectrograph, showed many bands belonging to the important $O_2(b\text{-}X)$ Atmospheric Band emission system. Previous ground-based measurements have shown only a single band, from the lowest vibrational level of the emitting state. Of particular interest is the fact that at the 762-nanometer position of the *b-X* 0-0 band, where earlier studies have shown only absorption features, these results showed both absorption at the $^{16}O^{16}O$ line positions and well-resolved emission at the positions of many of the $^{18}O^{16}O$ and $^{17}O^{16}O$ lines. These findings show that substantial advances can be made in understanding atmospheric emission phenomena by the use of astronomical tools.

The terrestial nightglow is the emission in the upper atmosphere, typically in the 80- to 100-km altitude region, originating from chemical reactions and atom recombination processes. As seen from the ground, this emission is dominated by two emitting molecular species, O_2 and OH. The OH emission is in the ground-state Meinel band system, beginning near 520 nm and extending well into the infrared region (*1*). O_2 emission is found in the near ultraviolet from the Herzberg band systems (*2*) and in the red and infrared from the Atmospheric ($b^1\Sigma_g^+ - X^3\Sigma_g^-$) and Infrared Atmospheric ($a^1\Delta_g - X^3\Sigma_g^-$) Band systems (*3*, *4*). The $a^1\Delta_g$ and $b^1\Sigma_g^+$ states of oxygen are the first and second electronically excited states, respectively (*5*). The region between 600 and 900 nm has typically been considered to be almost barren of molecular oxygen emission features, but we show here that with sufficient resolution and sensitivity, this region contains a great deal of spectral information, pertaining to atmospheric energy flow and the photochemistry of O_2, that can be extracted from ground-based observation.

As many astronomical measurements are made in the 600- to 900-nm region, considerable effort has been expended in accurately determining the positions of the ubiquitous

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