Microbes have a remarkable ability to synthesize nanostructures with precise control over their composition and functional properties. Understanding and exploiting these microbial activities will result in potentially transformative approaches to synthesizing nanomaterials for semiconductor, optical, and photoactive applications. Here we use diascopic and bright field microscopy to investigate the mechanism through which Shewanella species produce arsenic sulfide nanomaterials. The eventual aim is to synthesize industrially significant nanostructures.

**Biogenic Nanomaterials**

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**Shewanella and Arsenic Sulfide**

*Shewanella* species are known to reduce a variety of terminal electron acceptors, including thiosulfate and arsenate. Our laboratory uses strains MR-1 and ANA-3, as well as a variety of ANA-3 mutants. Microscopy experiments using ANA-3 attempt to characterize fiber elongation and nucleation processes.

**Proposed Pathway of As-S Nanofiber Production**

**Experimental Design**

*Shewanella* ANA-3 is incubated under anaerobic conditions with 20 mM lactate as the electron donor and 10 mM thiosulfate as the electron acceptor, in the presence of 5 mM sodium arsenate heptahydrate (Na$_2$HAsO$_4$·7H$_2$O). Fiber formation in the chamber is then monitored using time lapse diascopic and bright field microscopy.

**Ongoing Work**

Data revealing the composition of the nucleation points is necessary for complete characterization of the fiber formation process. To this end, NanoOrange protein stain will be used to locate all proteins present in the culture, thereby determining cell location relative to fibers. In addition, SEM images will be taken of just-formed nucleation points, shedding light on the local environment that allows fiber growth to begin.

**Experimental Analysis**

(A) Photograph of bright yellow macroscopic sheets of As$_x$S$_y$ that precipitated from solution. (B) SEM image of the sheets composed of nanofibers. (C) A higher magnification SEM image showing individual nanofibers.

**Bright Field Microscopy**

Room temperature growth of arsenic sulfide nanofibers as observed with bright field microscopy. Scale reads 1 µm. Geometry of other fiber clusters indicated growth from the fiber tip, suggesting abiotic crystallization. Fiber growth rates were measured both in 30 minute increments (time dependent) and as global averages.

**Time Dependent Growth Rate**

Binned averages of room temperature growth rate of more than 50 arsenic sulfide nanofibers. Decay in growth rate over time suggests a possible dependence on concentration of arsenic and sulfur available in the solution. Growth rate remains roughly constant after hour 21.

**Average Growth Rate**

Histogram of average room temperature growth rates, taken from a selection of nanofibers. Blue lines represent the subset of all data (orange) beginning in the first half of the experiment. Average growth rates were 2.92 nm/min (blue) and 3.76 nm/min (orange).

**Industrial Significance**

We are currently studying the ability of *Shewanella* strains (such as MR-1, which can respire selenite and tellurite) to produce arsenic selenide or arsenic telluride nanofibers. Since our findings suggest that arsenic incorporation into nanofibers may be an abiotic process, we may be able to replace the arsenic with other elements such as cadmium or zinc. This may provide a scalable method to efficiently produce a variety of semiconductor nanostructures, including arsenic selenide (which exhibits an optomechanical effect), cadmium selenide (used in quantum dots and laser diodes), and cadmium telluride (used in solar cells).

**Summary**

*Shewanella* sp. strain ANA-3 can produce macroscopic quantities of As-S nanofibers at room temperature at a rate of approximately 3 nanometers per minute. Data suggest biotically influenced nucleation and abiotic elongation processes.

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