Behavior of Shewanella Oneidensis MR-1 in a Sulfur and Zinc-Rich Medium and its Applications for Biosensing and Biomaterials

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Introduction

Shewanella oneidensis MR-1 is a dissimilatory metal-reducing bacterium (DMRB) that can grow under either aerobic or anaerobic conditions. When grown anaerobically, *S. oneidensis* is capable of reducing certain metal ions, such as Fe(III), Mn(IV) [1], and As(V), as well as sulfur compounds such as thiosulfate [2] and sulfite [3] by using them as terminal electron acceptors in its metabolic processes. *S. oneidensis* accomplishes this using redox-active c-type cytochromes which are present on the exterior of its cell walls. It is also known to extrude bacterial nanowires under conditions of electron acceptor limitation, and these nanowires contain conductive chains of c-type cytochromes that facilitate long-range electron transfer. [4] Through these mechanisms, *S. oneidensis* can also engage in direct electron transfer with an electrode, allowing voltammetric measurements of its electrochemical properties to be performed. [5]

Prior research has demonstrated the ability of *S. oneidensis* to precipitate zinc sulfide nanoparticles through thiosulfate reduction in a zinc-enhanced medium. [6] Zinc sulfide is notable in that it is also a semiconductor with a 3.54eV direct band gap (in its cubic form), and therefore is of value in photonics as well as other electronics applications. In our current research, we seek to expand on what is currently known about zinc sulfide precipitation via *S. oneidensis* metabolic processes. In order to do this we have cultivated *S. oneidensis* in a range of zinc-rich media and characterized the precipitates generated as well as performing voltammetric measurements on electrochemical systems containing *S. oneidensis* growing in zinc-enchanced media.

Materials and Methods

Shewanella oneidensis MR-1 culture was obtained from the American Type Culture Collection (ATCC) and grown in a chemically-defined medium consisting of the following: deionized water, PIPES buffer (3mM), ammonium chloride (10mM), potassium chloride (1.34mM), potassium phosphate monobasic (4.4mM), potassium sulfate (1.34mM), lactic acid as an electron donor (20mM) and 10mM of trace vitamin, mineral and amino acid solutions. The pH was adjusted to 7.0 using concentrated HCl and NaOH. The solution was sealed in 150mL culture bottles with 50mL solution and 100mL air, allowing cultures to grow aerobically. Culture bottles were then sterilized using an autoclave. Bottles were inoculated with 1mL culture and allowed to grow for 2-7 days such that biofilm growth was visible. This culture was then used to inoculate various secondary media as described in the subsequent sections.

For SEM imaging, samples were first placed in a solution of sterile MR-1 media (prepared as described above) and 2.5% glutaraldehyde. Samples were soaked in solution overnight, then placed in a series of deionized water and ethanol solutions with concentrations of 10%, 25%, 50%, 75% and 100%. Finally, samples were dried using an Autosamdri critical point dryer and then sputtered to a thickness of 1nm with either gold-palladium alloy or platinum.

For cyclic voltammetry measurements, a polished steel working electrode was soaked in 100% ethanol overnight, soaked in 6M HCl for 3 hours, and then dried overnight in a 100°C oven. Squares of Sigma-Aldrich SmartPor porous alumina were attached to the working electrode using copper tape, and the working electrode was sputtered with 1nm platinum to increase conductivity. The reference electrode was a Gamry Ag/AgCl standard working electrode. The counter electrode consisted of polished titantium wire. Cyclic voltammetry measurements were performed using a Gamry Reference 3000 potentiostat.

Effect of UV Light on Nanomaterial Precipitation

S. oneidensis was cultivated in 4 identical batches using a medium identical to the one described in the Methods section but with the addition of 15mM sodium thiosulfate, no potassium phosphate monobasic, and with slabs of graphite wool inside the bottles. Culture bottles were bubbled with 100% nitrogen for 30 minutes prior to sterilization to remove dissolved oxygen. Bottles were incubated at 30°C and rotated at 100rpm. After 48h of incubation, sterile zinc sulfate was added to each bottle to a concentration of 5mM. One set of bottles (Batches 3 and 4) were placed in a sterile hood on the UV light sterilization setting for 30 minutes both immediately after inoculation and immediately after addition of zinc sulfate. The other set of bottles (Batches 1 and 2) received no UV radiation. Bottles were permitted to incubate for an additional 96h after the addition of zinc sulfate, then the graphite was removed and treated for SEM imaging.

When imaged using SEM, the non-irradiated samples exhibited *S.oneidensis* bioflm growth and clusters of nanoparticles could be seen precipitating on and near the biofilm. Energy dispersive spectroscopy (EDS) indicated that the nanoparticle composition was consistent with zinc sulfate in Batch 2 and with a mixture of zinc sulfate and zinc sulfide in Batch 1.

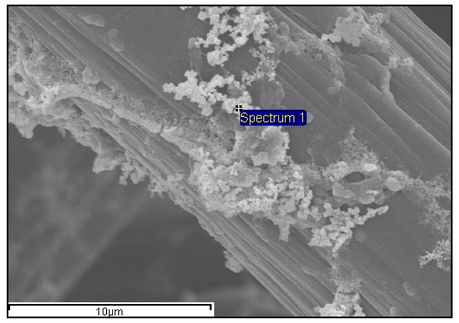


Figure 1: In this SEM micrograph, nanoparticles can be seen forming in the vicinity of *S. ondeidensis* bacteria. The indicated point contained 71% carbon, 9% oxygen, 10.5% sulfur and 9.1% zinc as measured by EDS.

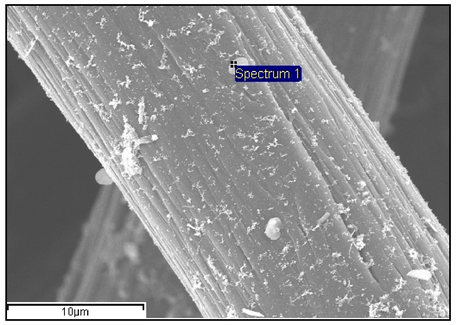


Figure 2: An SEM Micrograph from Batch 3 showing some residue but little to no visible bacteria or nanoparticles.

Replacement of Thiosulfate with Sulfite

Four additional batches were prepared using methods identical to those used for Batches 1-4 above except that sodium sulfite was used in place of sodium thiosulfate. In sulfite batches with no UV irradiation, limited biofilm growth was visible but zinc/sulfur nanoparticles were absent. In UV-irradiated sulfite batches, biofilm growth was also absent.

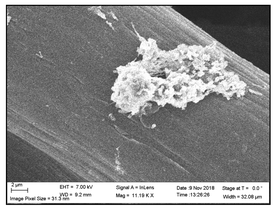


Figure 3: In the sulfite medium, small biofilm growths were visible but with no nanoparticles present.

Cyclic Voltammetry

In another experiment, *S.oneidensis* was cultivated in a jar containing a working, reference and counter electrode. The medium was identical to the one described in the Methods section but with the addition of 5mM sodium thiosulfate, 5mM zinc sulfate and 30mM PIPES buffer instead of 3mM. Jar was permitted to incubate at room temperature for 7 days, then the current through the counter electrode was measured as the working electrode voltage relative to the reference electrode was cycled between -1V and +1V over the course of several cycles. This was done at multiple scan speeds in order to determine the dependence of current/voltage behavior on scan rate. Saw a peak consistent with literature that disappeared.

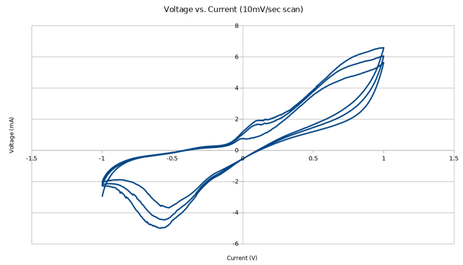


Figure 4: At a scan rate of 10mV/sec, asymmetrical redox peaks at -500mV are consistent with those found for c-type cytochromes in *Shewanella* in prior experiments. [7]

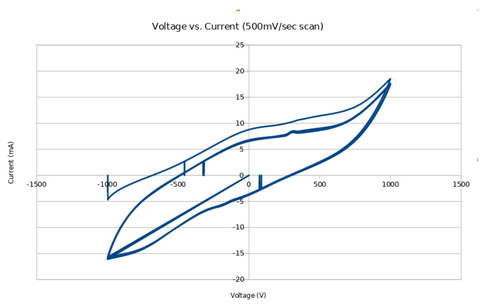
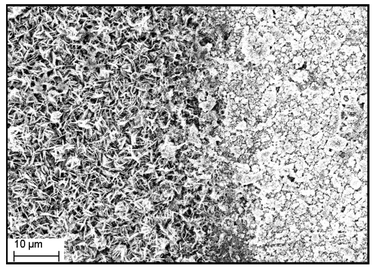


Figure 5: At a scan rate of 500mV/sec,-500mV/sec redox peak disappears.

Integration with Microporous Membranes

After voltammetric measurements were complete, SmartPor membranes were removed and imaged using SEM. Signficant crystal nucleation was visible on the microporous surface of the membrane, and EDS analysis indicated that the composition of the crystals was zinc phosphate. *S. oneidensis* bacteria could be see growing among the zinc phosphate crystals. At the edges of the membrane the alumina became amorphous with grains on the order of a few microns, and *S. oneidensis* could also be seen growing between these grains.

Figure 6: Edge of SmartPor membrane shows the nanoporous region (left) covered with zinc phosphate crystals and the edge region (right) consisting of amorphous alumni and no zinc phosphate crystals. *S. oneidensis* could be seen in between features on both sides.



Research Conclusions

- The choice of sulfur source has an effect of the precipitation of zinc sulfate by *S. oneidensis* in a medium enhanced with sulfur and zinc. Thiosulfate is more effective for zinc nanoparticle production than sulfite.
- Biofilm growth and nanoparticle production are halted by the introduction of UV light.
- When *S. oneidensis* is cultivated via batch culture and permitted to form a biofilm in a liquid medium, the behavior of the culture may vary even between batches with identical media recipes, necessitating multiple trials in order to fully understand results.
- In a zinc and sulfur-enchanced medium, *S. oneidensis* facilitates the precipitation of nanomaterials whose chemical composition is different from that of the reactants originally added to the medium.
- Cyclic voltammetric analysis indicates redox peaks in a system of *S. oneidensis* zinc and sulfur-enchanced media that are highly dependent on scan rate.
- *S. oneidensis* is capable of colonizing microporous surfaces consisting of either zinc phosphate or alumina.

Future Work

- Use transmission electron microscopy to confirm identity of biomaterials generated
- Exploration of the light sensitivity of biogenerated ZnS nanoparticles using spectrophotometry
- Attempt to characterize AC current/voltage behavior of electrode using electrochemical impedance spectroscopy
- Experiment with additional microporous substrates (etched silicon, quartz)
- Experiment with additional metal ions (lead, cadmium, silver)

Acknowledgement

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