A Biological Production of ZnS (Sphalerite) Nanoparticles with Desulfovibrio gigas

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Abstract

Sulfur-/sulfate-reducing bacteria (SRB) have been used for remediation of metal-contaminated ground water and soil through the precipitation of metal sulfides. These metal sulfides range from 3 nm to 3 μm in diameter. Of specific interest to this research are zinc sulfide nanoparticles (ZnS NPs) produced by SRBs with a diameter of ~3 nm. These ZnS NPs can be used as catalysts to grow nano-wires, and these nano-wires, coupled with green fluorescent protein (GFP), might be used to make a cost-efficient biosensor. Currently, there is no report of a pure culture that produces ZnS NPs; therefore, this study investigates the ability of a pure culture of *Desulfovibrio gigas* in two different media (Media 63 and Media 149) to produce ZnS NPs as a metal precipitate. This study shows that both media are able to grow *D. gigas* in the absence of zinc in the solution. *D. gigas* growth in media containing zinc could not be determined due to ZnOH precipitates.

Introduction

Sulfur/sulfur reducing bacteria (SRB) are a family of bacteria that can take in soluble metals and produce insoluble metal precipitates. Many researchers have examined bacterial systems because they are relatively simple systems. SRB have been used in the remediation of flooded zinc mines in Tennyson, Wisconsin (Labrenz et al., 2000.) Researchers have exploited the anaerobic nature of the SRB to filter out the contaminated soils and water. Specific to this research, *Desulfovibrio gigas* is an obligate anaerobe that produces hydrogen sulfide as a by-product of energy production. Instead of using oxygen as the terminal electron acceptor *D. gigas* uses sulfate. The sulfate in return reacts with hydrogen and forms H_2S. H_2S in solution is highly reactive with many metal ions and forms an insoluble metal precipitate. These precipitates can be filtered out and hence remediate the water contaminated by the metal ions(Labrenz and Banfield, 2004.) This research looks at exploring this process to acquire the insoluble metal precipitates and use it as a catalyst to make nano-wires. This research explores allowing zinc ions to react with the H_2S and forming ZnS nanoparticles (NPs). These ZnS NPs will in turn be used as a catalyst to make ZnO nano-wires. In contrast, other studies have used a mix culture of SRBs to produce ZnS NPs, but this study examines the feasibility of using a pure culture of *D. gigas* to produce ZnS NPs.

Materials and Methods

D. gigas was acquired from Christopher House, Assistant Professor in Earth and Mineral Department at the Pennsylvania State University. The stock culture provided by Christopher House was grown in media 149. It was white and cloudy with bacterial growth and contained tiny black particles. Under normal conditions, the bacteria would be incubated for 4 days and sub-cultured once a week. For this study, *D. gigas* was stored in the refrigerator until the media for ZnS NPs were ready.

Growth media (Media 149) for *D. gigas* was obtained from the DSMZ.de culture collection website (http://DSMZ.de) which contains trace minerals, vitamins, and various sulfates. For the purpose of this work, a few changes were made to the formulation of this media. Since this media is only a growth media for *D. gigas*, it did not contain a zinc source for the ZnS nanoparticles, therefore, $ZnSO_4$ were added to the media. A base medium containing common ingredients for all the samples was prepared in a 500-mL media bottle which was capped with a rubber septum and a cap with a hole, about ½ in diameter, drilled in the center. The medium was degassed with N_2 gas in the fume hood for 15 minutes. A 1.5-in needle was placed into the hole in the cap of the media bottle to feed in the N_2 gas and another needle was inserted adjacent to the previous needle to allow excess gas to vent out. The medium was dispensed in five 100-mL serum bottles and capped with a rubber septum and sealed with an aluminum seal in an anaerobic chamber. Varying concentrations of $ZnSO_4$ were dispensed into the five media bottle; they were Zn-free (Sample A), 0.005g/mL (Sample B), 0.002g/ml (Sample C), 0.001g/ml (Sample D), and 0.01g/ml (Sample E.) These concentrations were arbitrarily picked to find a approximate level of zinc that is toxic to the bacteria. After the addition of zinc, they were subsequently removed from the anaerobic chamber and placed in a fume hood. In the fume hood, 1.5-in needles were placed into the serum bottle through the rubber septum, and they were flushed using 20% N_2 gas and 80% CO_2 gas followed by pulling a vacuum for three cycles. The media were autoclaved for 20 minutes in liquid cycle at 121 oC.

In contrast, another media formulation (Media 63) was obtained from the DSMZ website. This media was used to grow a mixed culture of SRB to produce ZnS NPs. This medium was prepared using the same technique to make Media 149. The only difference between the two media was the ingredients. Media 63 did not contain vitamin solutions and trace mineral solutions added to Media 149, so the same concentration used to make Media 149 was used. Five different samples with varying concentration of $ZnSO_4$ were made. They were Zn-free (Sample 1), 0.005g/mL (Sample 2), 0.002g/ml (Sample 3), 0.001g/ml (Sample 4), and 0.01g/ml (Sample 5.)

All ten media were inoculated with 2 mL of *D. gigas* and allowed to incubate at 30^oC for 4 days. The inoculation syringe needle was flamed 4 times and inserted immediately into a 100-mL serum bottle which contained a stock N_2 gas. The N_2 gas was used to create an anaerobic atmosphere in the syringe. The needle was flamed 3 times and inserted into the stock culture of *D. gigas* in a 100-mL serum bottle and 2 mL of *D. gigas* was transferred to the fresh media.

The medium was dispensed in five 100-mL serum bottles in an anaerobic chamber. The five 100-mL serum bottles were flushed using 20% N_2 gas and 80% CO_2 gas and vacuumed for three cycles. Five media with varying concentration of $ZnSO_4$ were made; Zn-free (Sample A), 0.005g/mL (Sample B), 0.002g/ml (Sample C), 0.001g/ml (Sample D), and 0.01g/ml (Sample E). The media were autoclaved after adding the $ZnSO_4$. These concentrations were used to test for the zinc concentration that is toxic to the bacteria.

Another media formulation (Media 63) was obtained from the DSMZ website. This media was shown to be able to grow a mixed culture of SRB and produce ZnS NPs. The medium was dispensed into five 100-mL serum bottles in an anaerobic chamber. The five 100-mL serum bottles were flushed three times for 3 minutes using 20% N_2 gas and 80% CO_2 gas with a vacuum being applied in between flushing. For this media a varying concentration of $ZnSO_4$ was added; Zn-free (Sample 1), 0.005g/mL (Sample 2), 0.002g/ml (Sample 3), 0.001g/ml (Sample 4), and 0.01g/ml (Sample 5). In addition to the $ZnSO_4$, the vitamin solution and trace mineral solution used in Media 149 were added in an equivalent amount.

All ten media were inoculated with 2 mL of *D. gigas* and allowed to incubate at 30^{o}C for 4 days. The needle of a sterile syringe were flamed and inserted into the stock N_2 gas to create an anaerobic atmosphere in the syringe. The needle is flamed again and inserted into the stock solution of *D. gigas* and 2 mL of *D. gigas* is transferred to the media previously made.

Results and Discussion

Media 63

After the media was made Zn(OH)2 precipitate formed in the media. A simple test of combining different media components showed that zinc reacts with hydroxide ions in the solution to form $Zn(OH)_2$ at above pH 6. This caused a potential problem to this research because the normal growth media for *D. gigas* required a pH of 7.8. The media was prepared regardless of the problem and the bacteria were inoculated. Media 63 had a pH of about 6.3 and was slightly pinkish in color before inoculation. Some of the samples containing zinc had traces of precipitate. Five days after inoculation, a significant amount of bacterial growth was observed at the bottom of the serum bottles. The bacterial growth was viscous and looked gelatinous in texture; apparently growing as a biofilm as it resembled a membrane. Upon shaking the bottle several times, the membrane-like structure tore off from the bottom rather then it being re-suspended. Sample 1 had the most noticeable growth of this membrane like structure. In addition, Sample 1 had the most bacterial growth. Growth in Sample 2-5 could not be visually affirmed due to the presence of precipitate prior to inoculation. Bacterial growth of Media 63 containing zinc ions could not be determined. Further study is needed in using media with a lower pH to control the formation of $Zn(OH)_2$ precipitates.

It was anticipated that Media 63 might not support *D. gigas* growth due to the slightly acidic environment. The optimal growth pH for *D. gigas* was expected to be around 7.8 based upon the media formulation acquired from DSMZ. When pH levels are outside the limited tolerable range, certain membrane-bound proteins may become denatured due to disruption of ionic interactions among the amino acids, leading to loss of protein function. However, *D. gigas* was shown to be more resilient than expected and appeared to grown significantly in this media despite initial acidic conditions. This is fortunate since the low pH is useful to control precipitation. Unfortunately, the pH was not monitored; therefore, it is not possible to know if *D. gigas* was able to grow in the media because it was able to alter its environment, or because the pH conditions for growth are versatile. It would be interesting to test in the future the initial pH range in which *D. gigas* can grow and monitor how that pH changes during subsequent growth.

Media 149

Prior to inoculation, Media 149 had a pH of about 7.8 and it was colorless but cloudy with zinc hydroxide precipitates except for Sample A. After five days of incubation, Sample A was no longer clear; it became cloudy with *D. gigas* growth. Growth of the other samples could not be determined due to the presence of the precipitate. The precipitate was determined to be zinc hydroxide by testing various media components for precipitate formation. A solution of zinc ions was observed to react with other media components and produce a precipitate.

In concluding this summer research, both media type showed that D. gigas growth was indeed possible in the absence of zinc ions. Further research is needed in using media with a more acidic pH to control precipitation. In addition, based on the premise that D. gigas did grow in media with zinc ions, this experiment should precede to the next step of growing ZnO nano-wires.

Literature Cited

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