Use of sulfidation as a novel method for reducing the toxicity of silver nanoparticle pollution

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Abstract

With increasing use of silver nanoparticles for their antimicrobial properties, greater amounts of nanoparticles are being released into the environment, with potentially adverse impacts on benign microorganisms. This project examined use of sulfidation as a viable approach to reduce the toxicity of silver nanoparticle pollution during wastewater treatment. Five sets of silver nanoparticle samples were used, keeping one set pure and as the control, and exposing the remaining four sets to increasing concentrations of sulfidation agent (Na_2S). Three toxicity indicators, namely nanoparticle size, surface charge and release of silver ions were measured. Lastly, the actual toxicity of samples was tested by measuring the change in E. coli population after exposing E. coli cultures to the five samples. The release of silver ions and surface charge decreased and nanoparticle size increased as sulfide concentration went up, indicating reduction in toxicity. As the sulfide concentration increased, the amount of E. coli cells killed decreased, proving that exposure to sulfide reduced toxicity. The experimental results supported the hypothesis, establishing that sulfidation is a potential solution for reducing the harmful effects of nanoparticle pollution.

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Key Words

Silver Nanoparticles, Silver Sulfide Nanoparticles, Silver Nanoparticle Pollution, Sulfidation, Wastewater treatment, Biotoxicity

Abbreviations and Acronyms

Ag-NP: Silver Nanoparticles
DLS: Dynamic Light Scattering
g: grams
ICPMS: Inductively Coupled Plasma Mass Spectrometer
mL: milliliter
mM: millimolar
nm: Nanometer
PVP: Polyvinylpyrrolidone
TEM: Transmission Electron Microscope

Acknowledgements

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Biography

My name is Anirudh Jain, and I am a freshman at Catlin Gabel School in Portland, Oregon. I have a lot of varied interests and participate in a broad range of extracurricular activities. I play tennis on my school's varsity team and play the piano at level 8. I am passionate about science and its application to real-life problems. Therefore, I actively participate in science fairs. I have won one of the two top category awards at Northwest Science Expo for three consecutive years. I have been one of 30 national finalists at the Broadcom MASTERS science fair two years in a row. I was also one of 15 finalists in my age group from the U.S. in the 2012 Google Science fair. A couple of years ago, I created a silver

nanoparticle based portable water filter because, on family trips to India and Guatemala, I saw that it was extremely difficult to get drinking water since the sources of natural water were contaminated with microbes. After I made the silver nanoparticle based filter, I was concerned that if someone were to throw the disposable filter away, silver nanoparticles would find their way into the environment and inadvertently harm microorganisms. I wanted a way to detect such pollution. So, last year, I experimented with using diatoms as bioindicators of silver nanoparticle pollution. This year, I wanted to explore ways to take care of this pollution, which is what my current project is about. I would love to continue to discover and invent as I pursue a career in science and technology.

Introduction

Many everyday products such as clothing and containers are coated with silver nanoparticles to take advantage of silver's antimicrobial properties. Literature study shows evidence that silver has been used as an antimicrobial agent since 3100 B.C (Vasilev, 2010). Recent advances in technology have made it possible to synthesize silver into nanoparticle form. In this form, it is far more effective than plain silver. A nanoparticle is a small object between 1 and 100 nanometers (1 billionth of a meter) that behaves like a whole unit (Berger, 2010). Nanoparticles react more effectively with substances than bigger particles of the same element because the ratio of surface area to volume is much higher due to their small size. The larger surface area is particularly relevant to adsorption scenarios where nanoparticles can very rapidly interact with the surrounding material (Vecios, 2006). The graph below depicts the relationship between size of nanoparticle and surface area.

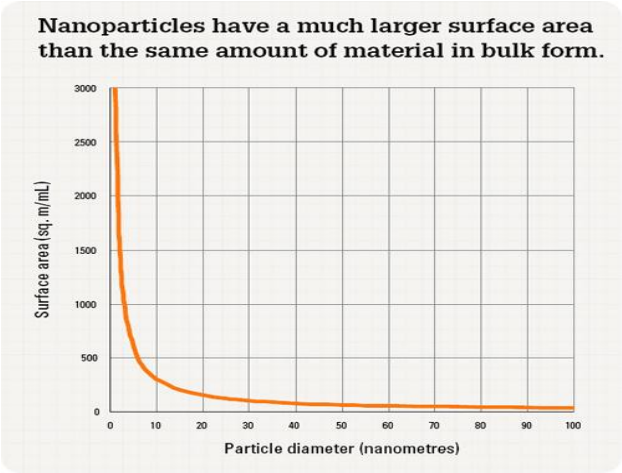

Figure 1: Relationship between nanoparticle size and surface area
(Nanotechnology and Food, 2009)

Silver acts against microorganisms by affecting the ability of the microbe to take in oxygen. It deactivates the enzymes upon which the microbe relies for oxygen. This destroys the cell membrane of the microbe, and takes away the ability of its Deoxyribonucleic acid (DNA) to make replicas or mutate (Vecios, 2006). The picture below depicts how the silver nanoparticles penetrate the cellular membrane to enter bacteria and inactivate their enzymes, thus killing the bacteria.

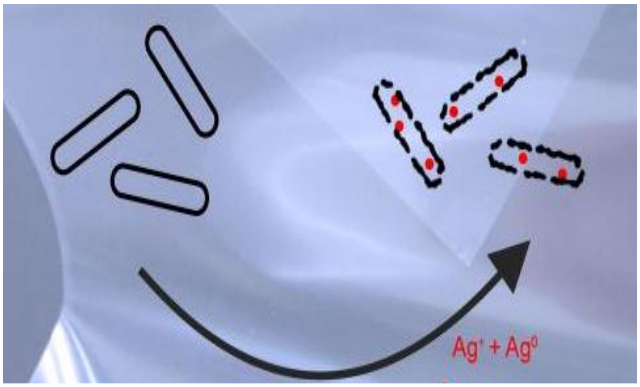

Figure 2: Ag-NP and cell interaction

(Swiss Federal Institute Of Technology, 2008)

As of now, no known pathogen, bacteria, fungus or virus has been able to develop immunity to silver nanoparticles. The antimicrobial property of silver is amplified significantly in the nanoparticle form. The graph below shows effect of various concentrations of silver nanocolloids on survival rate of two common bacteria: "Staphylococcus capitis" and "Escherichia coli".

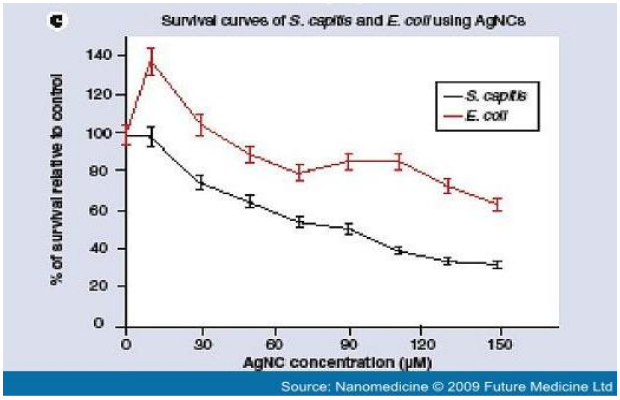

Figure 3: Effect of silver nanocolloid concentration on survival rate of "Staphylococcus capitis" and "Escherichia coli"

(Rehab M Amin, 2009)

Silver acts equally on all single-celled organisms regardless of whether they are harmful to humans or not. While this natural property makes it possible to use ionic silver to eliminate a broad range of harmful microorganisms, its indiscriminate action has far-reaching effects which can cause unintended harm to benign or beneficial bacteria. Presence of silver nanoparticles in soil matter can kill useful bacteria which play a key role in soil enrichment by breaking down organic matter (Hu, 2008). Modern wastewater treatment plants use beneficial bacteria to get rid of some contaminants. If these bacteria are exposed to silver nanoparticles, they will be eliminated, adversely affecting the treatment process and harming the local ecology. A study by O. Choi showed that silver nanoparticles are toxic to nitrifying bacteria and that they interfere with wastewater treatment methods (O. Choi, 2008). Effectiveness of the water treatment process would be compromised if the crucial nitrifying bacteria were to be killed. Therefore, with the increasing use of silver nanoparticles in consumer products, it has become extremely important to find a way to neutralize the toxicity of these particles during the wastewater treatment process.

Many studies have demonstrated the toxicity of silver nanoparticles. However, most of these toxicity studies have not considered the transformations that silver nanoparticles may undergo in different environments. Finding a method to reduce toxicity of Ag-NP pollution needs an understanding of the relationship between toxicity and physical properties. A study proposed that the antibacterial activity of silver nanoparticles depends on their size (Sally D. Solomon, 2007). When Ag-NPs are small (less than 20 nm), the release of silver ions dominates the antibacterial activity of nanosilver. This suggests that dissolved Ag species control part of the toxicity (Sally D. Solomon, 2007). Surface charge also plays a major role in determining the toxicity of silver nanoparticles. El Badawy et al. recently suggested that the surface charge of silver nanoparticles, which was controlled by different capping agents (PVP, citrate, and polyethyleneimine), is responsible for their difference in toxicity with a more positive surface charge resulting in greater toxicity (Amro M. El Badawy, 2011).

Kim et al. discovered the presence of Ag_2S nanoparticles in sewage sludge products even in areas where there were no industrial sources of silver nanoparticles (Kim, Park, Murayama, & Hochella, 2010). This indicated that (a) silver nanoparticles were coming from household wastewater and (b) the silver nanoparticles were reacting naturally with sulfides. If the silver sulfide nanoparticles were less toxic than silver nanoparticles, sulfidation would be a promising method of treating silver nanoparticle pollution.

Therefore, the objectives of this study were to (1) synthesize silver nanoparticles, expose the samples to varying concentrations of sodium sulfide, and measure impact of sulfidation on toxicity indicators such as size, surface charge and release of silver ions, and (2) directly test for toxicity by exposing E. coli populations to control and sulfidized samples, and measuring the extent to which the E. coli growth is adversely affected.

Materials and Methods

40 mL of silver nanoparticle solution was synthesized using 10.0 mL of 1. 0 mM Silver Nitrate, 30 mL of 2.0 mM Sodium Borohydride and PVP (Polyvinylpyrrolidone). A 10-mL volume of 1.0 mM silver nitrate was added drop wise (about 1 drop per second) to 30 mL of 2.0 mM sodium borohydride solution that had been chilled in an ice bath. The reaction mixture was stirred vigorously on a magnetic stir plate. The solution turned light yellow after the addition of 2 mL of silver nitrate and a brighter yellow when all of the silver nitrate had been added. The entire addition took about three minutes, after which the stirring was stopped and the stir bar removed. The resulting 40 mL of Ag-NP's was divided into 15 vials (3 groups of 5 samples). 1 sample in each group was used as the control and the other 4 samples were exposed to different concentrations of sulfide. Of the 3 groups, one group was used for Dynamic Light Scattering (DLS) and Inductively Coupled Plasma Mass Spectrometer (ICPMS) studies, one group was used for surface charge studies, and one group was for testing toxicity impact on E. coli.

Figure 4: Bacterial culture in a petri dish (left) and array of all petri dish samples (right) used to determine the toxicity of the control and sulfidized samples

The molarity ratio of S/Ag was planned to be 0.05, 0.1, 0.5, and 1 with the control as simply silver nanoparticles and no sulfide. 4 beakers were prepared with 100 mL of water and appropriate amount of sodium sulfide added to get solutions of 0.05, 0.1, 0.5 and 1.0 concentration. 3.3 mL from each beaker was added to the corresponding vial, resulting in 0, 0.05, 0.1, 0.5, and 1 as the molarity ratios for the samples.

Transmission electron microscopy (TEM) was used to characterize and confirm nanoparticles for both the control and sulfidized samples.

Malvern Zetasizer ZS90 was used for surface charge measurements. Three surface charge measurements per sample were taken to eliminate errors. Size measurements were conducted using a Dynamic Light Scattering (DLS) machine.

Five beakers with 200 mL of nanopure water were prepared, making sure that the beakers were sterile. Five lengths of dialysis tubing which only allow particles of size 10 nm or less to pass through were used. The dialysis tubing was soaked in water for 1 hour. Part of the dialysis tubing was rolled up and clamped with a dialysis clamp. The remaining tubing was slowly filled with 2 mL of one of the silver nanoparticle solutions. The top part of the tubing was rolled and clamped. This tubing setup was inserted into one of the beakers so that it was completely submerged. This was repeated for all the samples. The samples were covered with parafilm to prevent contamination. After a period of two days, the tubing was removed and the concentration of silver ions was measured using an Inductively Coupled Plasma Mass Spectrometer (ICPMS).

100 mL of Luria-Bertani medium was synthesized using 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 1 liter deionized water. The medium was autoclaved for 25 minutes at 120 degrees Celsius. The medium was inoculated with E. coli by using an inoculating loop. The cells were allowed to grow in the medium for one day. Twenty (5 groups of 4 – two before and two after) petri dishes were autoclaved as well as the agar. Agar was made using 7.5g agar powder, 5 g tryptone, 2.5 g yeast extract and 5 g NaCl. The plates were poured and allowed to cool. The medium was separated into five different experimental samples each with 5 mL. From each sample, 100 microliters was removed, placed on the corresponding petri dish, and spread with the inoculating loop. This was done twice per sample on separate plates. 500 microliters of each silver nanoparticle solution was added to the five samples of medium. Then 100 microliters of the sample was taken and placed on petri dish and spread with an inoculating loop. The shape of the resulting bacterial culture was traced on paper and cut out. The area of the paper was measured to determine the extent of growth of bacterial culture.

All of the data collected was analyzed for trends in surface charge, size and release of silver ions. Also, the toxicity of the silver nanoparticles was estimated by examining percentage of E. coli killed.

Results and Discussion

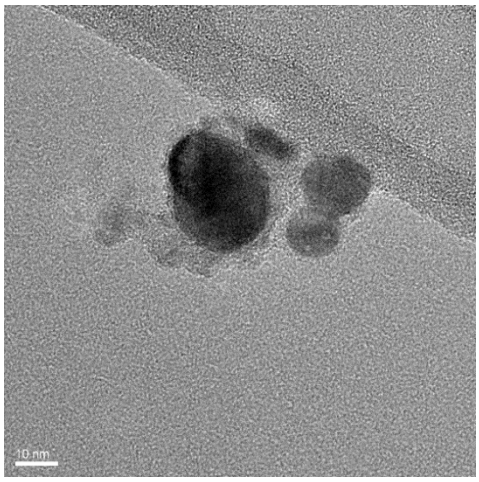

Figure 5: TEM image of nanoparticle

Transmission Electron Microscope (TEM) imaging was first used to confirm the presence of nanoparticles (control and sulfidized samples) after the synthesis process. Figure 5 shows a single nanoparticle in solution.

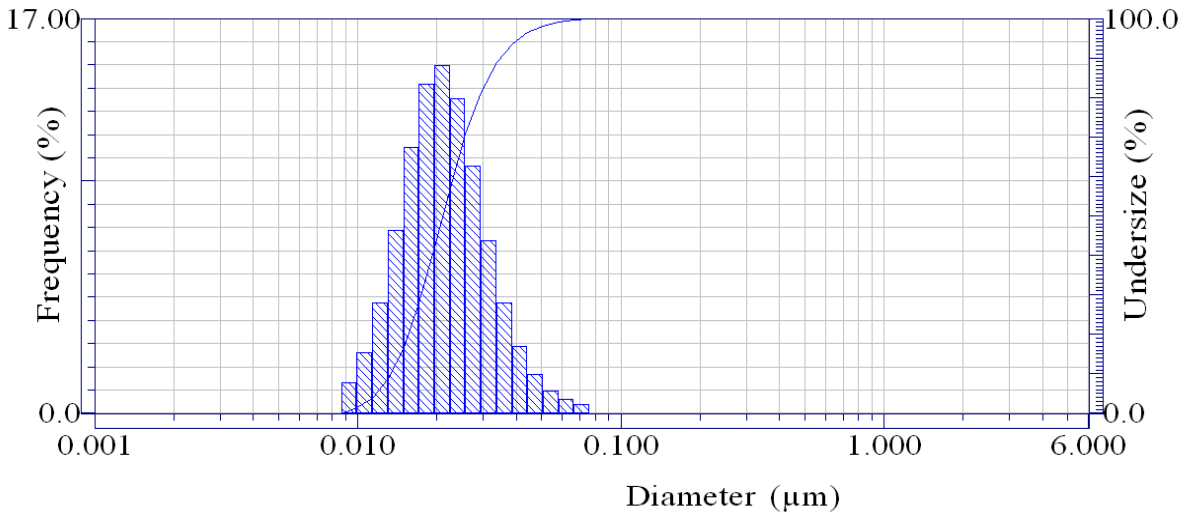

Figure 6: DLS nanoparticle size distribution

Figure 6 shows the size distribution of silver nanoparticles measured using Dynamic Light Scattering process. The particle size was normally distributed in a very small range, indicating that the synthesis process was well controlled.

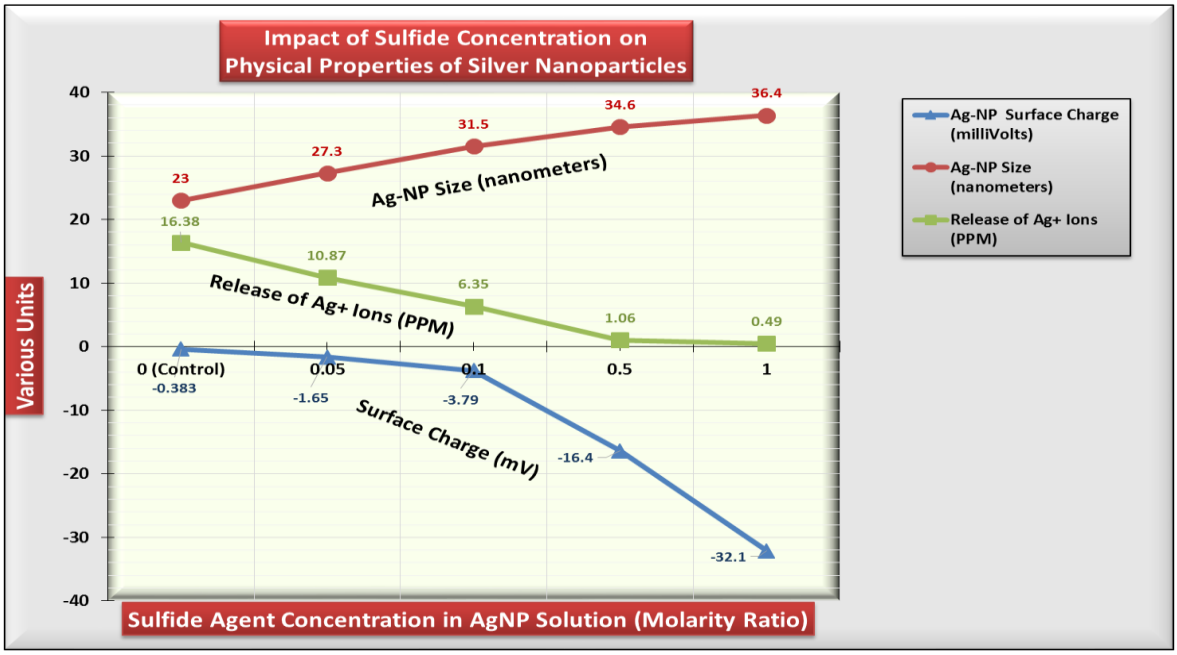

Figure 7: Impact of Sulfide Concentration on Properties of Silver Nanoparticles

Figure 7 illustrates the change in properties of silver nanoparticle solution with exposure to increasing concentrations of sodium sulfide, while all other factors were kept constant. The three properties measured as toxicity indicators were surface charge, release of silver ions, and size of silver nanoparticles. The changes in these three indicators of silver nanoparticle toxicity were clearly correlated to increased molarity ratio of sulfides to silver nanoparticles solution (0, 0.05, 0.1, 0.5, and 1.0). Exposure to higher concentration of sulfide caused silver nanoparticles to become larger, lose surface charge, and release fewer silver ions.

Smaller nanoparticles are more toxic due to a high surface area to volume ratio. An increase in size of nanoparticles results in reduction in toxicity since the surface area to volume ratio decreases. Similarly, lower surface charge and reduction in the ability to release silver ions reduces the ability of silver nanoparticles to kill microorganisms. Therefore, these results clearly indicate that there is an inverse correlation between sulfide concentration and toxicity indicators, i.e., toxicity decreases with an increase in sulfide concentration.

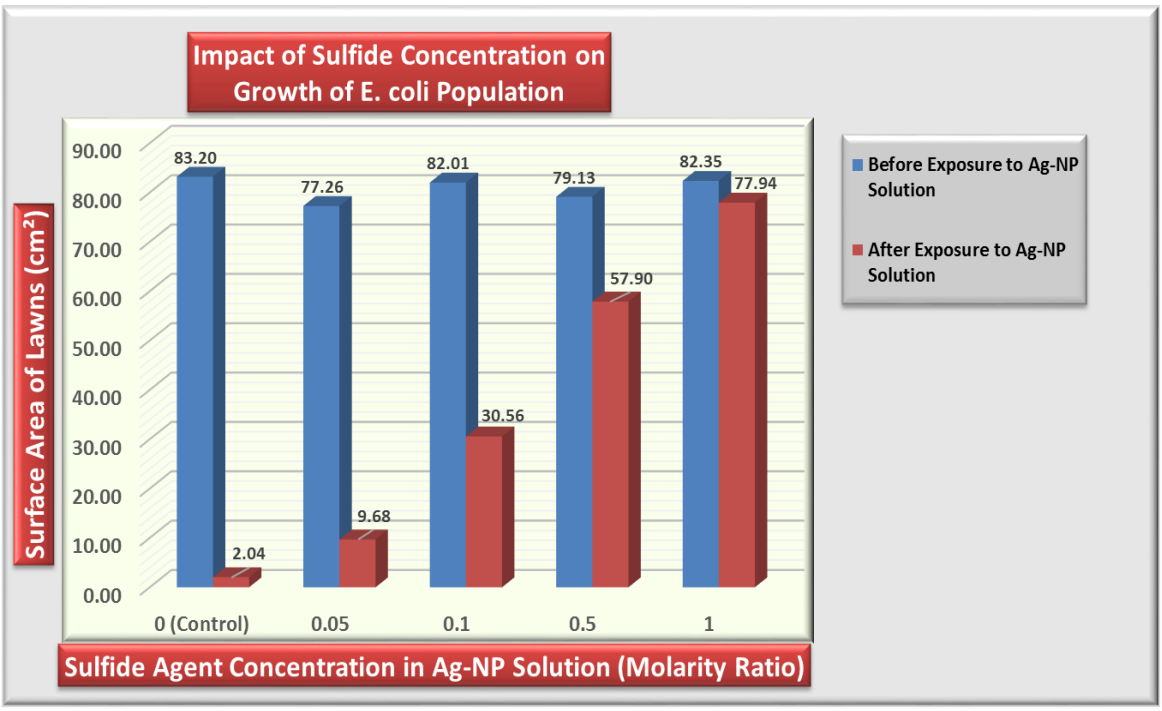

Figure 8: Impact of Sulfide Concentration on Growth of E. coli

Figure 8 depicts the cell growth of E. coli in five identical samples, measured before the samples were exposed to silver nanoparticles solutions with different concentrations of sulfide, and measured after the exposure. As the concentration of sulfide increased, the amount of E. coli killed decreased.

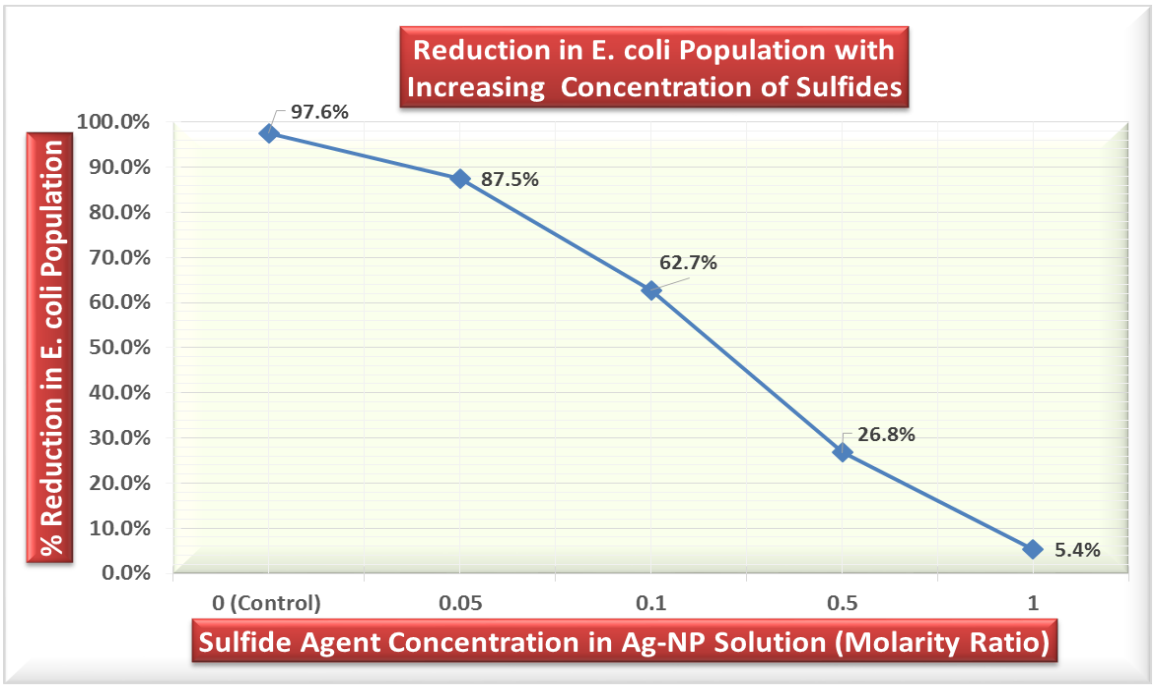

Figure 9: Reduction in E. coli Population with Increasing Concentration of Sulfides

Figure 9 presents the impact on E. coli in terms of percentage reduction in the population. There is a clear negative trend in the line, depicting that the exposure to higher concentration of sulfide causes silver nanoparticles to lose their ability to reduce growth of E. coli. The percentage decrease in E. coli populations drastically declines from 97.6% to 5.4% as the sulfide concentration increases from a molarity ratio of 0 to a molarity ratio of 1.0. This shows that the toxicity of the silver nanoparticles has been reduced by exposure to sulfide.

The experiment was well controlled so that only one independent variable was actively being changed, namely concentration of sodium sulfide. Care was taken to sterilize all equipment to avoid any contamination. Multiple readings were conducted on the same sample for better validation of results, and multiple samples were used. All of the materials were from one base sample or source. Statistical studies were done to determine the accuracy of the experiment by calculating the coefficient of variance. The coefficient of variance was between a reasonable range of 0.03 and 0.06 for all samples which indicates very low variation across samples.

In a wider scientific and social context, designing a practical process to treat silver nanoparticle pollution during wastewater treatment is a critical problem to solve due to the adverse impact this pollution has on the environment. As discussed, there is clear evidence of silver nanoparticles and derivatives being present in wastewater sludge even in the absence of significant industrial sources of silver nanoparticles (Kim, Park, Murayama, & Hochella, 2010). Numerous studies have conclusively established the indiscriminate lethal effect of silver nanoparticles on microorganisms. Research on this topic is in its infancy because the threat has become significant only recently due to an explosive growth in number of consumer products incorporating silver nanoparticles for their excellent microbial properties. Therefore, there is a strong desire to harness the beneficial properties of silver nanoparticles, while protecting earth's aquatic and terrestrial resources from its inadvertent harmful effects. This study is a step forward in solving this conundrum.

Even though there is an increasing awareness of the unintended consequences of manufactured nanoparticles being released into the environment, no definite measures have been deployed in wastewater treatment plants to combat this threat. Given the results of this study, it is clear that the novel approach of sulfidation has the potential to address this gap in the wastewater treatment process.

Conclusions and Future Work

1. As the concentration of sulfide in silver nanoparticle solution increases, the properties of silver nanoparticles which contribute to its toxicity (size, surface charge and release of silver ions), are affected so that the silver nanoparticles become less toxic.
2. The increase in nanoparticle size and decrease in release of silver ions, surface charge, and harmful impact on microorganisms are solely attributable to sulfidation.
3. The hypothesis that the toxicity of silver nanoparticles, as measured by the release of silver ions, surface charge, and harmful impact on microorganisms has an inverse correlation to the degree of sulfidation of silver nanoparticles, has been proven correct.
4. It is practical to employ sulfidation as a method to reduce the harmful impacts of silver nanoparticle pollution on the environment.
5. Sulfidation of wastewater should be done before the nitrification stage during wastewater treatment to reduce the ability of silver nanoparticles contained in wastewater from adversely affecting nitrifying bacteria.

Future Work

In order to convert this research to a working solution, the following questions should be researched:

1. Can this technique be used to reduce toxicity of other kinds of engineered nanoparticles (since this experiment only used silver nanoparticles)?
2. What environmental conditions can affect the interaction between sulfide and silver nanoparticles? Will the presence of other contaminants affect results in a material way?
3. Will interaction with gaseous compounds such as Hydrogen Sulfide (H_2S) be more effective than aqueous solution of sodium sulfide (Na_2S) used in the experiment?
4. Will the capping agent used for Ag-NP affect toxicity?
5. Are there some unintended environmental implications of using the sulfidation process?

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