Use of silver nanoparticles synthesised by *Bacillus subtilis* in antimicrobial water filtration

Romen Mahajan (3i3), Teo Chuan Kai (3i1)

Group 1-50

<u>Abstract</u>

Silver nanoparticles have shown potential in water purification in previous research. However, conventional methods of nanoparticle production can cause a great deal of pollution and are energy intensive, making them a less attractive option. Biosynthesis of silver nanoparticles, particularly using bacteria, has been explored in recent years as a potential alternative to conventional synthesis due to its significantly lower environmental impact and reduced energy usage. The aim of this study was to synthesise silver nanoparticles using *Bacillus subtilis* and investigate the effectiveness of silver nanoparticles synthesised by bacteria in water filtration. This was accomplished through immobilising the silver nanoparticles onto polyester foam, then using the foam in two separate tests; soaking the foam in water contaminated by *E. coli* and having water contaminated by *E. coli* pass through the foam in the form of a filter. The results were then compared to those obtained using conventionally-synthesised silver nanoparticles and sterilised water. It was found that bacteria-synthesised silver nanoparticles reduced the presence of *E. coli* in treated water to a similar extent to silver nanoparticles synthesised using the conventional method. For the filtration test, there was a 100% decrease in CFUs counted for both the bacteria-synthesised and conventionally-synthesised nanoparticle setups when compared to the sterilised water setup. This finding could have significant implications in the use of silver nanoparticles for water filtration through significantly decreasing the environmental and energy cost of silver nanoparticle production, and could be of significant use in the designing of low-cost, eco-friendly water filters.

<u>Introduction</u>

Literature Review

Nanoparticle research is currently an area of intense scientific study. Nanoparticles are materials that have dimensions of 1-100nm in diameter. They have a wide variety of potential applications in fields such as healthcare, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single electron transistors, light emitters, nonlinear optical devices, and photo-electrochemical area. (Güzel and Erdal, 2018)

One such application is water purification using silver nanoparticles due to their antimicrobial properties. The World Health Organisation estimates that 829 000 people die each year from diarrhoea as a result of unsafe drinking-water, sanitation, and hand hygiene. In addition, half of the world's population will be living in water-stressed areas by 2025, due to factors such as climate change, increasing water scarcity, population growth, demographic changes and urbanization. (WHO, 2019)

Silver nanoparticles have shown significant potential in water filtration. The absorbant capacity of silver nanoparticles embedded in chitosan, which is a non-toxic and biodegradable natural polymer (Saifuddin, Nian, Zhan, & Ning, 2011), using microwave irradiation toward Atrazine, a pesticide, is 0.5 mg g^{-1} of the adsorbant in 65 minutes. Two grams of cross-linked chitosan-silver nanoparticle composite microbeads were capable of removing 94% of Atrazine from aqueous pesticide solution (1.0 mg L^{-1}) (Saifuddin et al., 2011). This method serves as a convenient and cost-effective method of removing pesticides from drinking water.

Silver nanoparticle-coated polyurethane foam also demonstrated effectiveness as an antibacterial water filter. Using a prototypical water filter with a flow rate of 0.5 L/min, in which contact time was of the order of a second, the output count of *E. coli* was nil when the input water had a bacterial load of 105 colony-forming units (CFU) per ml (Jain & Pradeep, 2005). Combined with the low cost and effectiveness in its applications, the technology may have large implications to developing countries.

Graphene oxide (GO) nanosheets impregnated with silver nanoparticles (Ag NPs), fabricated by the in situ reduction of adsorbed Ag^{+} by hydroquinone (HQ) in a citrate buffer solution, exhibited strong antibacterial activity. The Ag NP/GO composites performed efficiently in bringing down the count of *E. coli* from 106 cfu/ml to zero with 45 mg/l GO in water. The micron-scale GO nanosheets (lateral size) enable them to be easily deposited on porous ceramic membranes during water filtration; making them a promising biocidal material for water disinfection (Bao, Zhang, & Qi, 2011).

There are many chemical and physical procedures for the synthesis of metallic nanoparticles. However, these methods have many problems such as the use of toxic solvents such as sodium borohydride, generation of hazardous by-products, and high energy consumption (Singh, Kim, Zhang, & Yang, 2016). Biosynthesis is an environmentally friendly method of producing metal nanoparticles. Several options have been explored for the production of metal nanoparticles, including plants, algae, fungi, viruses and bacteria (Singh et al., 2016).

The bacteria *Rhodopseudomonas capsulata* is capable of producing gold nanoparticles extracellularly that are quite stable in solution in an efficient, eco-friendly and simple process where the shape of the gold nanoparticles is controlled by pH (He, et al., 2007). Extracellular agents produced by newly isolated bacterial strains of *Bacillus pumilus, B. persicus,* and *Bacillus licheniformis* were used to synthesise silver nanoparticles in the size range of 77–92 nm. Silver nanoparticles were stable (zeta potential ranged from −16.6 to −21.3 mV) and showed excellent in vitro antimicrobial activity against important human pathogens (Elbeshehy et al., 2015).

Bacillus subtilis was found to be able to synthesise gold nanoparticles both intra and extracellularly (He et al., 2007), while being able to synthesise silver nanoparticles exclusively extracellularly. The gold nanoparticles were formed after 1 day of addition of chloroaurate ions, while the silver nanoparticles were formed after 7 days. The nanoparticles were characterized by X-ray diffraction, UV-vis spectra and transmission electron spectroscopy. X-ray diffraction revealed the formation of face-centered cubic (fcc) crystalline gold nanoparticles in the supernatant, broth solution and bacterial pellet. Silver nanoparticles also exhibited diffraction peaks corresponding to fcc metallic silver. UV-vis spectra showed surface plasmon vibrations for gold and silver nanoparticles centered at 530 and 456 nm, respectively. TEM micrographs depicted the formation of gold nanoparticles intra- and extracellularly, which had an average size of 7.6 ± 1.8 and 7.3 ± 2.3 nm, respectively, while silver nanoparticles were exclusively formed extracellularly, with an average size of 6.1 ± 1.6 nm (He et al., 2007).

Another study also found that silver nanoparticles (AgNPs) can be synthesised using bacterial strains of *Bacillus subtilis,* as well as *Listeria monocytogenes* and *Streptomyces anulatus*. This study also showed that it has fungicidal and larvicidal properties. This shows that *Bacillus subtilis* is a viable option to develop eco-friendly AgNPs (Soni & Prakash, 2014).

Objectives

- Synthesise silver nanoparticles using *Bacillus subtilis*
- Immobilise silver nanoparticles in polyester foam
- Investigate the effectiveness of synthesised silver nanoparticles in water filtration

Hypothesis

Coating polyester foam with silver nanoparticles synthesised by *Bacillus subtilis* will reduce presence of *E. coli* in treated water to a similar extent to polyester foam coated with conventionally produced silver nanoparticles.

Materials and Methods

Materials:

- Silver nitrate ($AgNO_3$)
- Trisodium citrate ($Na_3C_6H_5O_7$)
- *Bacillus subtilis*, ATCC 19659
- *Escherichia coli*, ATCC 25922
- LB agar
- LB broth
- Polyester foam

Apparatus:

- UV-vis spectrophotometer
- Autoclave
- Orbital Shaker Incubator
- Centrifuge
- Plastic Funnels
- Petri dishes

i) Synthesis of silver nanoparticles using *B. subtilis*

B. subtilis was plated on LB agar medium and incubated overnight. 25 ml of LB broth were added to 100 ml conical flasks, which were then autoclaved. The *B. subtilis* was inoculated into the broth, and then placed in an orbital shaker under controlled agitation for incubation. After 24h, the cultures were centrifuged and the supernatants (free of any kind of precipitates) passed through sterilized membranes of 0.2 μm pore diameter before being used as catalysts for AgNPs synthesis. 20 ml of the supernatant was placed in a flask, and silver nitrate was added to an eventual concentration of 1 mM. Finally, the whole setup was incubated in an orbital shaker.

ii) Synthesis of silver nanoparticles using conventional method

25 ml of 0.005 M stock solution of silver nitrate was diluted in water to 125 ml at 0.001M and then heated until it began to boil. 5 ml of 1% sodium citrate solution was added, and the solution was heated until it became pale yellow, and then cooled to room temperature.

iii) Coating polyester foam with silver nanoparticles and preparing filter

Polyester foam was sterilised in the autoclave, then divided into three pieces soaked in bacteria-synthesised silver nanoparticle solution, conventionally-synthesised silver nanoparticle solution and sterilised water respectively overnight. The pieces were then washed repeatedly with water to remove any adsorbed ions like citrate, and air-dried. Each piece of foam was then divided in half, and half of the foam was packed into plastic funnels to make filters for filtration test, with the remaining half reserved for the soaking test.

iv) Colony Count Test (soaking)

10 ml of LB broth was inoculated with *E. coli* and incubated overnight in a shaking incubator, then poured into test tubes. 1 cm x 9 cm x 0.6 cm pieces of both silver nanoparticle-coated foams as well as the uncoated foam were placed into separate test tubes. After 10 minutes, the foam pieces were squeezed to release treated water. Water was then extracted from the test tube and 10 μL of it was plated at $x10^{-4}$ dilution using LB agar and plate pour method. The plates were incubated for 24 hours and observed the next day for growth of *E. coli* and counted for the number of Colony Forming Units (CFUs).

v) Colony Count Test (filtration)

A bulk solution of *E. coli* ATCC 25922 in water was prepared, of which 10 ml was diluted to 150 ml. 50 ml of the solution was passed through each previously prepared filter, and the water that passed through the filters was collected after 10 minutes. 10 μL of the treated water was plated at $x10^{-2}$ dilution using LB agar and plate pour method. The plates were incubated for 24 hours and observed the next day for growth of *E. coli* and counted for the number of CFUs.

Results and Discussion

Soaking Test

16 CFUs were counted in the bacteria-synthesised silver nanoparticle solution setup (Figure 1), 255 in the conventionally-synthesised silver nanoparticle solution setup (Figure 2) and 482 in the sterilised water setup (Figure 3). There was a 96.7% decrease in CFUs counted from the sterilised water setup to the bacteria-synthesised nanoparticle solution setup, and a 47.1% decrease in CFUs counted from the sterilised water setup to the conventionally-synthesised nanoparticle solution setup to the sterilised water setup, indicating that both had antibacterial qualities (Figure 4).

Figure 1: Soaking test result for bacteria-synthesised silver nanoparticle solution setup, visible colonies labelled with marker

Figure 2: Soaking test result for conventionally-synthesised silver nanoparticle solution setup, visible colonies labelled with marker

Figure 3: Soaking test result for sterilised water setup, visible colonies labelled with marker

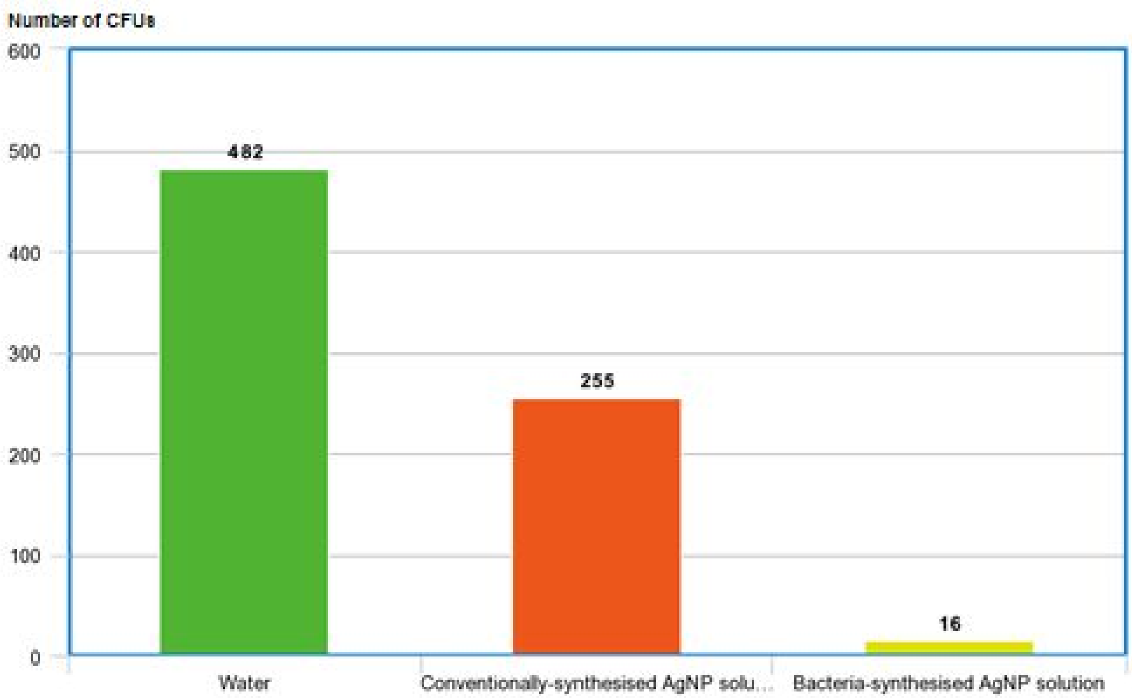

Figure 4: Soaking test results

There was a significant decrease in CFUs counted when comparing the sterilised water setup to both silver nanoparticle setup. Additionally, the bacteria-synthesised silver nanoparticle solution setup had fewer CFUs than the conventionally-synthesised silver nanoparticle solution setup, which would suggest that the bacteria-synthesised silver nanoparticles had stronger antibacterial qualities than the conventionally-synthesised.

Filtration Test

394 CFUs were counted in the sterilised water setup (Figure 7), whereas 0 CFUs were counted in both the bacteria-synthesised silver nanoparticle solution setup (Figure 5) and the conventionally-synthesised silver nanoparticle solution setup (Figure 6).

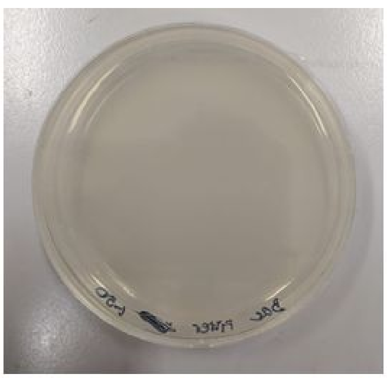

Figure 5: Filtration test result for bacteria-synthesised silver nanoparticle solution setup, visible colonies labelled with marker

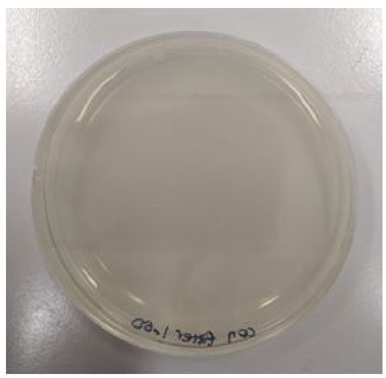

Figure 6: Filtration test result for conventionally-synthesised silver nanoparticle solution setup, visible colonies labelled with marker

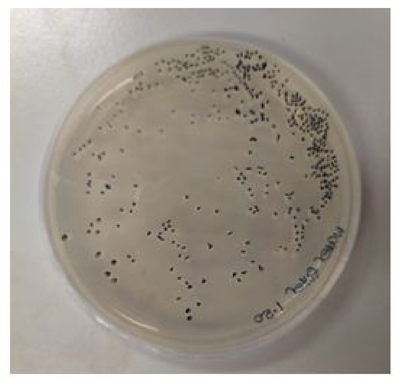

Figure 7: Filtration test result for sterilised water setup, visible colonies labelled with marker

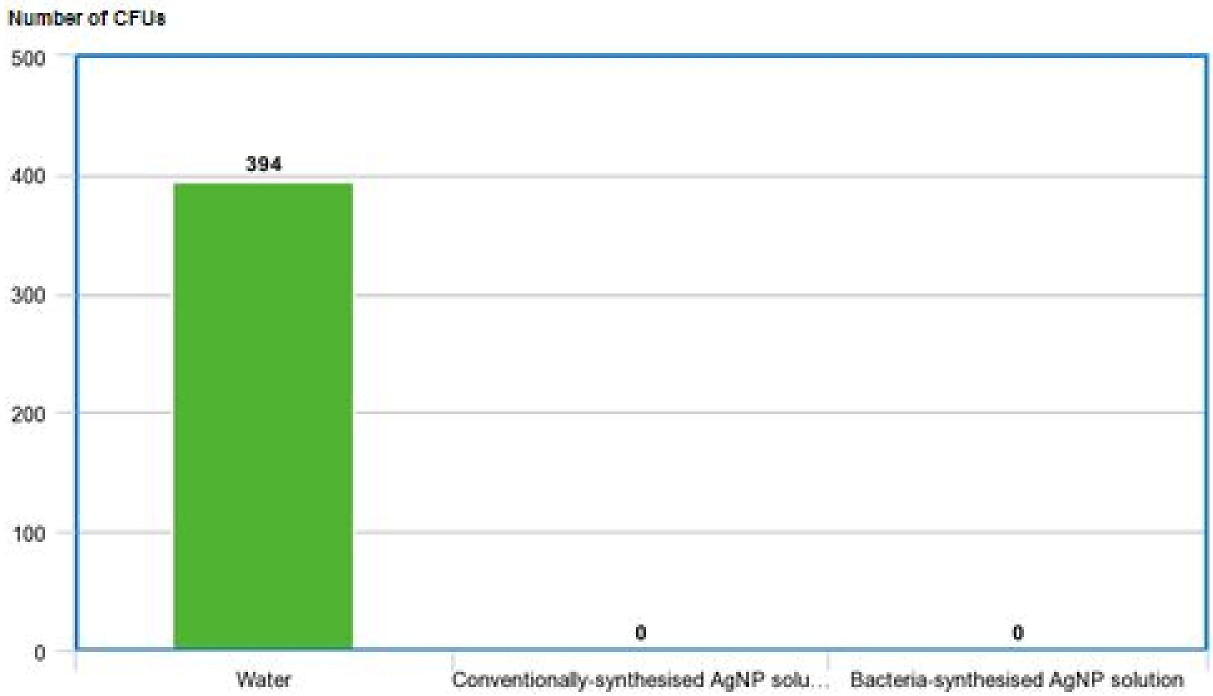

Figure 8: Soaking test results

The results indicate that 100% of *E. coli* cells were killed in both the bacteria-synthesised silver nanoparticle solution setup and the conventionally-synthesised silver nanoparticle solution setup (Figure 8), suggesting that bacteria-synthesised silver nanoparticles have antibacterial properties similar to that of conventionally-sythesised silver nanoparticles.

<u>Conclusion and Future Studies</u>

Conclusion

Silver nanoparticles were successfully synthesised using *Bacillus subtilis*, and showed similar physical properties to conventionally-synthesised silver nanoparticles, such as the solution becoming yellow. The silver nanoparticles were also successfully immobilised onto polyester foam for use in water purification. The results of the two experiments both indicated that bacteria-synthesised silver nanoparticles exhibit similar antibacterial qualities as conventionally-synthesised silver nanoparticles, with the bacteria-synthesised silver nanoparticles solution setups showing a significant decrease in number of CFUs of *E. coli* counted, comparable to or greater than that of the conventionally-synthesised silver nanoparticles setup. Of particular relevance is the filtration experiment as, when applied in a filter, the bacteria-synthesised silver nanoparticles solution setup removed 100% of *E. coli* from the contaminated water, thus suggesting it would have high effectiveness as a water filter used to treat water. The results suggest that bacteria-synthesised silver nanoparticles would be of significant use in cheap and environmentally friendly water filters.

Future Studies

In a true water filtration situation, far greater quantities of water would be passed through the bacteria-synthesised silver nanoparticles filters, and over a longer period of time. Hence, the capability of bacteria-synthesised silver nanoparticles to remain immobilised on a filter and maintain effectiveness as greater volumes of water pass through it should be looked into. It also may be worth studying the effectiveness of bacteria-synthesised silver nanoparticle filters in filtering pesticides, heavy metals and other contaminants, as well as whether silver nanoparticles synthesised by bacteria can be used as a substitute for conventionally-synthesised silver nanoparticles in other applications as well.

Bibliography

Bao, Q., Zhang, D., & Qi, P. (2011). Synthesis and characterization of silver nanoparticle and graphene oxide nanosheet composites as a bactericidal agent for water disinfection. Journal of Colloid and Interface Science, 360(2), 463–470. doi: 10.1016/j.jcis.2011.05.009

Elbeshehy, E. K. F., Elazzazy, A. M., & Aggelis, G. (2015). Silver nanoparticles synthesis mediated by new isolates of Bacillus spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus and human pathogens. Frontiers in Microbiology, 6. doi: 10.3389/fmicb.2015.00453

Encyclopedia Britannica (2020) nanoparticle | Definition, Size Range, & Applications https://www.britannica.com/science/science

He, S., Guo, Z., Zhang, Y., Zhang, S., Wang, J., & Gu, N. (2007). Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata. Materials Letters, 61(18), 3984–3987. doi: 10.1016/j.matlet.2007.01.018

Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638. doi: 10.1039/c1gc15386b

Jain, P., & Pradeep, T. (2005). Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnology and Bioengineering, 90(1), 59–63. doi: 10.1002/bit.20368

Microchem Laboratory(2016) Bacillus subtilis https://microchemlab.com/microorganisms/bacillus-subtilis

Parikh, R. Y., Singh, S., Prasad, B. L. V., Patole, M. S., Sastry, M., & Shouche, Y. S. (2008). Extracellular Synthesis of Crystalline Silver Nanoparticles and Molecular Evidence of Silver Resistance from Morganella sp.: Towards Understanding Biochemical Synthesis Mechanism. ChemBioChem, 9(9), 1415–1422. doi: 10.1002/cbic.200700592

Que, Z. G., Torres, J. G. T., Vidal, H. P., Rocha, M. A. L., Pérez, J. C. A., López, I. C., … Hernández, J. S. F. (2018). Application of Silver Nanoparticles for Water Treatment. Silver Nanoparticles - Fabrication, Characterization and Applications. doi: 10.5772/intechopen.74675

Raveendran, P., Fu, J., & Wallen, S. L. (2003). Completely “Green” Synthesis and Stabilization of Metal Nanoparticles. Journal of the American Chemical Society, 125(46), 13940–13941. doi: 10.1021/ja029267j

Reddy, A. S., Chen, C.-Y., Chen, C.-C., Jean, J.-S., Chen, H.-R., Tseng, M.-J., … Wang, J.-C. (2010). Biological Synthesis of Gold and Silver Nanoparticles Mediated by the

Bacteria Bacillus Subtilis. Journal of Nanoscience and Nanotechnology, 10(10), 6567–6574. doi: 10.1166/jnn.2010.2519

Saifuddin, N., Nian, C., Zhan, L., & Ning, K. (2011). Chitosan-silver Nanoparticles Composite as Point-of-use Drinking Water Filtration System for Household to Remove Pesticides in Water. Asian Journal of Biochemistry, 6(2), 142–159. doi: 10.3923/ajb.2011.142.159

Singh, P., Kim, Y.-J., Zhang, D., & Yang, D.-C. (2016). Biological Synthesis of Nanoparticles from Plants and Microorganisms. Trends in Biotechnology, 34(7), 588–599. doi: 10.1016/j.tibtech.2016.02.006

Soni, N., & Prakash, S. (2014). Antimicrobial and mosquitocidal activity of microbial synthesized silver nanoparticles. Parasitology Research, 114(3), 1023–1030. doi: 10.1007/s00436-014-4268-z

Thakkar, K. N., Mhatre, S. S., & Parikh, R. Y. (2009). Biological synthesis of metallic nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine, 6(2), 257–262. doi: 10.1016/j.nano.2009.07.002

World Health Organisation (2019) Drinking Water

https://www.who.int/news-room/fact-sheets/detail/drinking-water