Effective Disinfection of *Escherichia coli* Contaminated Water Using Silver Nanoparticle-Decorated Magnetic Cobalt Cores

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Abstract

In recent years, the development of silver nanoparticles (AgNPs) and their application in wastewater treatment have emerged as highly effective disinfection methods. Wastewater treatment processes effectively remove silver particles and colloids (most processes exceed 95%), but this still leaves notable concentrations that escape to effluent-receiving waters. To address this challenge, in this study, novel magnetic nanocomposites, silver nanoparticle-decorated magnetic cobalt (AgNPs/Co), were studied for disinfection of water contaminated with Escherichia coli (E. coli). First, the magnetic efficiency of the synthesized nanocomposites was measured. To measure the disinfection efficiency of AgNPs/Co in E. coli-contaminated water, various studies have used concentrations ranging from 10 to 50 μg. The results demonstrated an impressive antibacterial efficiency rate of 99.6% when using AgNPs/Co. Additionally, the efficiency rate of collecting the novel magnetic nanocomposites was found to be 100% using a magnet. The AgNPs/Co technology not only exhibits highly efficient water purification capabilities, but also offers the added benefit of complete removability using a magnet, a simple yet effective collection method. This feature plays a crucial role in preventing the introduction of toxic AgNPs into reservoirs, which could negatively impact both human populations and ecosystems. By enabling the production of clean water while preserving the environment, this technology provides an innovative solution for wastewater treatment.

Keywords: *Silver nanoparticles, Cobalt cores, Polyol method, Disinfection, Escherichia coli, Wastewater*

1. INTRODUCTION

In the absence of adequate wastewater treatment, there is a persistent risk of diseases such as cholera, typhoid, hepatitis A, and polio spreading[1]. Currently, chlorination, ultraviolet (UV) radiation, and ozone are the most commonly used disinfection methods for US onsite wastewater treatment systems. However, repeated use of chlorine has caused the evolution of chlorine-resistant bacteria, which are not properly filtered out before the water is returned to the environment.[2] UV radiation, which is effective against specific microorganisms, has a limited scope. Additionally, ozone treatment fails to target the microorganisms' DNA and arginine, an essential amino acid, which can facilitate horizontal gene transfer, thereby exacerbating public health risks.[3] It is crucial to develop alternative and more efficient methods for wastewater treatment to address these challenges and to ensure the protection of public health and the environment.

Silver nanoparticles (AgNPs) are regarded as highly effective disinfectants for wastewater treatment because of the antibacterial mechanisms of silver ions (**Figure 1**). The silver ions released from nanoparticles disturb the permeability of the cell membrane upon attachment to the bacterial wall.[4–6] After entering the cell, the ions 1) bind to sulfhydryl proteins, leading to protein inactivation,[7] 2) interfere with the respiratory chain, causing oxidative stress,[8–10] and 3) disrupt DNA replication, resulting in lipid damage. Eventually, the bacterial wall and membrane break as a result of cytoplasmic leakage, destroying the bacteria. AgNPs inactivate over 99% of Escherichia coli (E. coli) bacteria after several seconds of contact.[11]

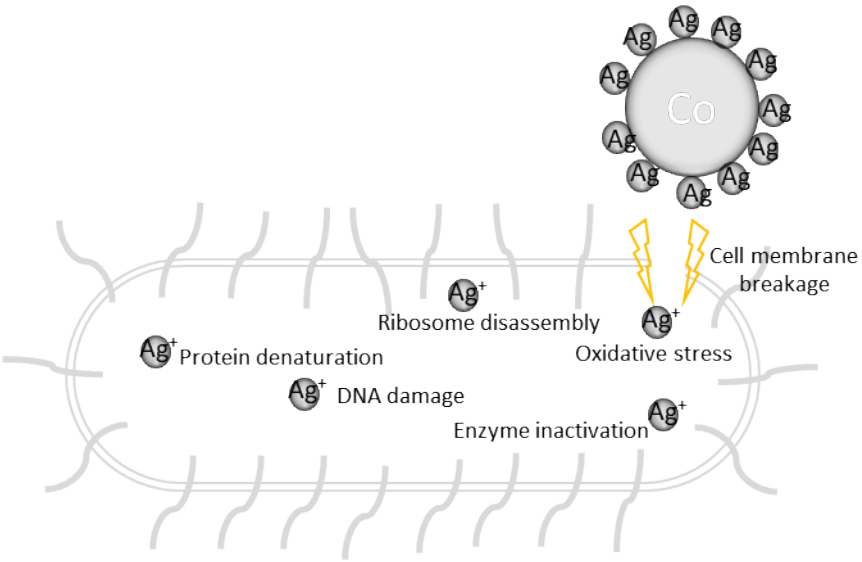


Figure 1. Mechanisms for antimicrobial actions of silver nanoparticle-decorated magnetic cobalt.

The widespread use of AgNPs in commercial and professional applications has resulted in the regular ingestion of residual silver, estimated to be within the range of 20–80 micrograms (µg), through sources such as water contamination, dietary supplements, and food packaging.[12,13] In order to mitigate potential health risks associated with silver exposure, the Environmental Protection Agency limits the daily exposure to silver to 5 µg per kilogram (kg) per day, which is approximately 310 µg for the average human adult weighing 62 kg. AgNPs, upon binding to various human tissues, can elicit toxic effects, including cell activation that leads to the generation of reactive oxygen species, inflammation, and ultimately cell death.[12] Additionally, several reports have documented instances of skin discoloration attributed to the toxicity of AgNPs.[14–16] The absence of effective AgNPs collection not only poses a risk to human health, but also impacts soil communities and aquatic systems.[17–21]

Although AgNPs have been proven to be an effective bactericide in water treatment methods, the recollection of leached silver remains a challenge.[22] This research aimed to assess the efficiency of using silver nanoparticles (AgNPs) in the bacterial removal of *E. coli* from a contaminated water test solution and to explore the potential of using magnets to recollect AgNPs. In this research, wastewater was defined by adding E. coli into the water matrix. In order to recollect AgNPs using a magnet, silver nanoparticle-decorated magnetic cobalt (AgNPs/Co), was synthesized by the polyol method, which is a widely-used cost-effective and facile soft chemical nanoparticle synthesis method that has been proven to be scalable for industrial application and effective in preparing core-shell nanostructures with tailored size and shapes.[23] In this study, a modified polyol process with a transmetalation reduction method was adopted from Kanwal, et al., (2019) and used to synthesize the desired silver nanoparticle coated cobalt core (Kanwal et al., 2019).[24] This method allowed the fabrication of silver onto the cobalt core medium and control of the nanoparticle size. In contrast to the referenced article, this research extended the boundaries of exploration by evaluating the removal efficiency of nanoparticles through magnetic means. This study also probed the potential implications of silver introduction into wastewater treatment plants, specifically addressing concerns related to silver poisoning. By giving the nanoparticles a magnetic property, the AgNPs/Co can be recollected using magnets, a quick yet promising method of preventing the silver from leaching into the environment.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals, including cobalt acetate tetrahydrate, silver acetate, ethylene glycol, polyvinylpyrrolidone (PVP), and hydrazinium hydroxide were purchased from Sigma Aldrich. As reference, commercial silver nanoparticles (50,000 ppm in a 5 mL solution, diameter: 15nm) (US Research Nanoparticles, US7160) were purchased. Nutrient agar (Innovating Science, IS5350) purchased from Innovating Science was used for the antibacterial tests. *E. coli* (Carolina Biological, #155065) was purchased from Carolina Biological. An incubator (VEVOR, XHC-25) was purchased from Vevor to let the *E. coli* cultures grow during the antibacterial tests. Neodymium N52 magnets were used as the recollection material. The N52 magnets were purchased from totalElement (totalElement, B1X12X14N52-5PK).

2.2. AgNPs/Co Synthesis

Figure 2 shows that the synthesis of nanocomposites was carried out in two stages. First, the cobalt cores were synthesized by dissolving ethylene glycol in a precursor solution of cobalt acetate.[25] Hydrazine hydrate was the reducing agent, and PVP was the stabilizing agent. The reaction was carried out in a hot plate with constant stirring at 200 rpm with a stir bar. A solution of 0.4 milliliter (mL) of hydrazine hydrate and 25 mL of ethylene glycol was added dropwise to the 25 mL precursor solution under constant stirring at room temperature. A 50 mL polymer solution was prepared by dissolving PVP into ethylene glycol. This solution was added and stirred for 15 minutes with an overhead stirrer to prevent the cobalt cores adhering to the magnetic stir bar. The solution was heated to 195° C and the temperature was maintained for 30 minutes. 100 µg of silver acetate was mixed at 200 rpm for 15 minutes. The solution was then heated to 120° C and kept for 15 minutes under continuous stirring. The finalized nanocomposites were collected

with magnets and then washed three times to remove impurities. The AgNPs/Cos were precipitated in an aqueous solution until tested.

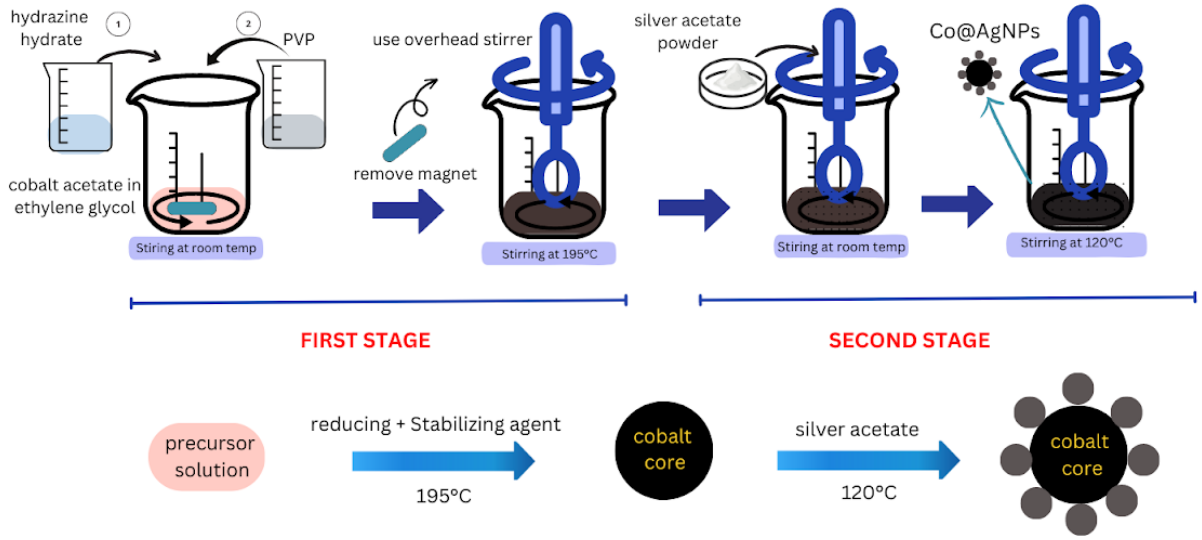


Figure 2. Schematic of Cobalt Core Silver Two-step Process of Nanocomposite Preparation.

2.3. AgNPs/Co Characterization

The AgNPs/Co size was examined with a Phenom Pharos desktop field emission scanning electron microscope (FE-SEM) using a secondary electron detector (SED) from Thermo Scientific (Waltham, MA, USA).

2.4. Disinfection Tests of *E. coli* Contaminated Water

Antibacterial properties were evaluated by the inactivation of bacterial cells on the surface of agar plates via the well diffusion method. To model *E. Coli* contaminated water, *E. coli* dilutions were created by adding 9 mL of distilled (DI) water (Snugell CPAP distilled water) and 1 mL of *E. coli*. Further serial dilutions were carried out until a total dilution factor of 10^{-6} was reached as the synthetic wastewater bacterial concentration.

For each experiment, a concentration of 10 mg of AgNPs/Co was used for the antibacterial activity tests. The commercial silver nanoparticle solution was diluted to match this concentration. For comparison, 0.2 mL of the commercial silver nanoparticles were diluted with 0.8 mL of distilled water for a final concentration of 10 mg. 0.25 mL of *E. coli* solution was evenly spread with a cell spreader on the top of nutrient agar plates. The petri dishes were kept in an incubator at 37° C for 24 hours.

To find the zone of inhibition, an *E. coli* solution of 10^{-3} was spread on the plate. Concentrations of 10,000 µg/mL of both the AgNPs/Co and commercial silver were then added equidistant to each other on the agar plate. The sensitivity of the different concentrations of nanocomposite and the commercial silver nanoparticles to the *E. coli* were determined by the clear zone around the respective samples and the diameters were measured in millimeters (mm).

To determine the relationship between reaction time, antibacterial efficiency of AgNPs/Co, and *E. coli* neutralization, further experiments were carried out by manipulating the serial dilutions of the E. coli broth. The experiments involve exposing the *E. coli* to four different dilutions of the synthesized nanoparticles for varying time intervals (1, 3, 5, and 10 minutes). The reaction time experiments aimed to identify the optimal duration for the nanoparticles to effectively deactivate the *E. coli*. Additionally, before being dispersed onto nutrient agar-filled plates for further analysis, the *E. coli* suspension is centrifuged for 5 minutes, which helps separate the bacterial cells from the surrounding medium. To ensure the reliability of the results, all tests were conducted in triplicate, meaning each experiment is repeated three times.

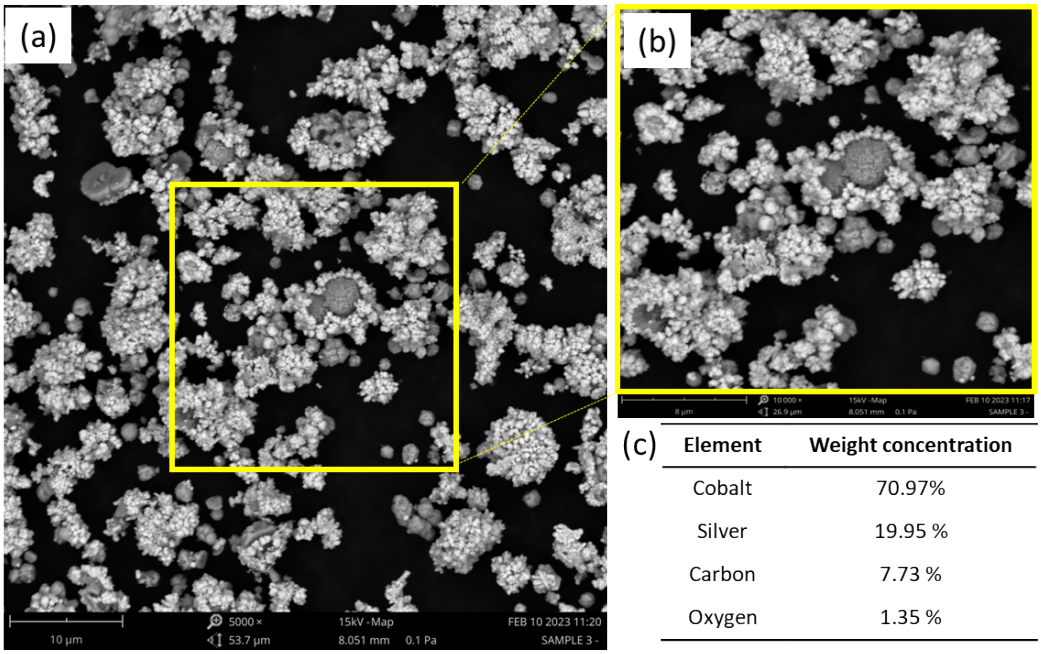
To evaluate the antibacterial efficiency of AgNPs/Co at various concentrations in neutralizing *E. coli*, the correlation between the concentration of AgNPs/Co, the deactivation of *E. coli*, and the concentration of silver acetate used during the nanocomposite synthesis were

investigated. Three different concentrations of the AgNPs/Co (10 mg/mL, 20 mg/mL, 30 mg/mL) and three different concentrations of the silver acetate (50 mg, 100 mg, 200 mg) were tested in triplicate.

3. RESULTS AND DISCUSSION

3.1. AgNPs/Co Characterization

By examining the field emission scanning electron microscope (FE-SEM) images (**Figure 3**), the hypothesis that silver nanoparticles will grow successfully on the surface of cobalt core particles was proven (**Figure 3 (a) and (b)**). SEM micrographs show spherical shape and 3–5 mm size range of nanocomposites. This confirms that the first portion of the two-step process **(Figure 2)** creates the cobalt core, and the second portion creates the silver nanoparticle decorations.



(c)

Element	Weight concentration
Cobalt	70.97%
Silver	19.95 %
Carbon	7.73 %
Oxygen	1.35 %

Figure 3. (a, b) Field emission scanning electron microscope (FE-SEM) image of AgNPs/Co nanocomposite synthesized; (c) Energy dispersive spectroscopy (EDS) result of present elements in the nanocomposite sample.

SEM micrographs showing the spherical morphologies and size range of nanocomposites 3-5 µm energy dispersive spectroscopy (EDS) results show that the mixing ratio of silver and cobalt in the synthesized nanocomposite particles is around 2:8 (**Figure 3 (c)**). The silver nanoparticles can also be observed to have a size around 1-2 µm, which is significantly less than the commercial silver nanoparticles, which had a size of around 15 µm. The size of the nanoparticles can be assumed as one of the largest factors in the difference of the efficiency between the AgNPs/Co and the commercial AgNPs.

These findings provide strong evidence for the successful growth of silver nanoparticles on the surface of cobalt core particles, resulting in the formation of spherical nanocomposites with a specific size range and a precise mixing ratio of the two components.

3.2. Commercial AgNPs versus AgNPs/Co

The disinfection rate of all synthesized AgNPs/Co and commercial AgNPs against tested microorganisms (*E. Coli*) was determined using a diffusion method. **Figure 4** presents images that present the antibacterial activity of 10 mg/mL and 50 mg/mL of the synthesized AgNPs/Co and commercial AgNPs. Both agar plate samples produced a perimeter of bacterial disinfection; however, the AgNPs/Co had a larger perimeter than the commercial AgNPs. The inhibition zones of the AgNPs/Co were 22 mm and 26 mm for the concentrations 10 mg and 50 mg, respectively.

The inhibition zones of the commercial AgNPs were 11 mm and 16 mm for the concentrations 10 mg and 50 mg, respectively.

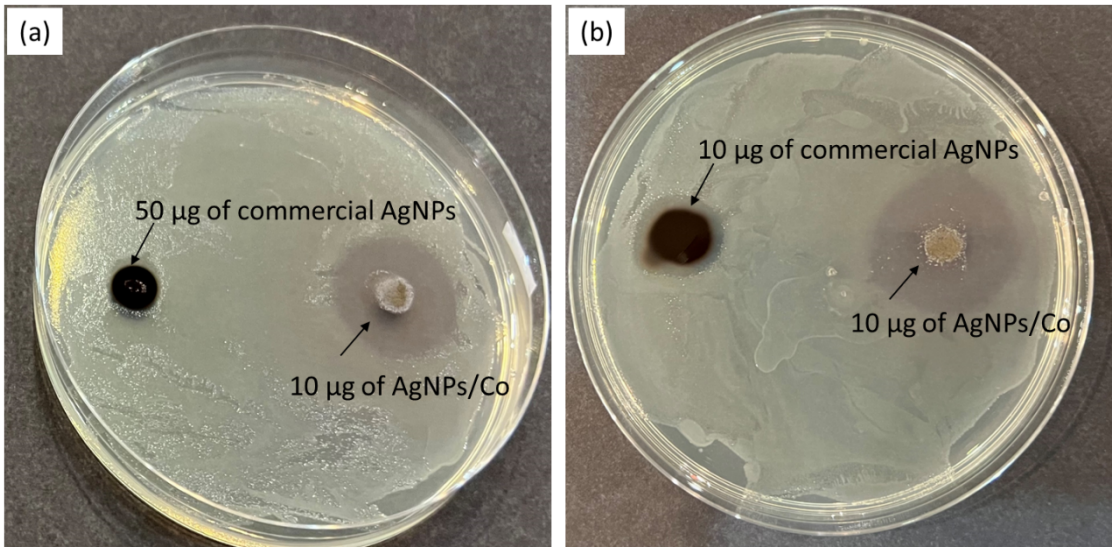


Figure 4. Comparison of the antibacterial efficiency of the commercial silver nanoparticles and cobalt core silver nanoparticles. (a). Side by side comparison of a single dot application of commercial silver nanoparticles (0.05 g/mL) and 1 mL of AgNPs/Co (0.01 g/mL) in an agar plate containing *E. coli*. (b). Side by side comparison of 100 µL of commercial silver nanoparticles (0.01 g/mL) and 1 mL of AgNPs/Co (0.01 g/mL)

3.3. Magnet Efficiency Test

To investigate the magnetism of AgNPs/Co, a comparison was made with commercial silver nanoparticles, both dispersed in water at a concentration of 0.01 gram (g)/mL. **Figure 5** clearly demonstrates that the AgNPs/Co exhibit a reaction to the magnet, whereas the commercial silver nanoparticles do not. Subsequently, a magnet efficiency test was conducted to determine the nanoparticles' removability by magnets. In this test, 0.2 g of the AgNPs/Co were added to 50 mL of water. After stirring the solution for 3 minutes to ensure full suspension of the nanocomposites, a magnet was employed to collect the AgNPs/Co. These nanocomposites were gathered separately

and left for 24 hours to dry. Prior to analysis, the weight of the nanoparticles was measured before and after removal from the aqueous suspension to verify their effective extraction by the magnet.

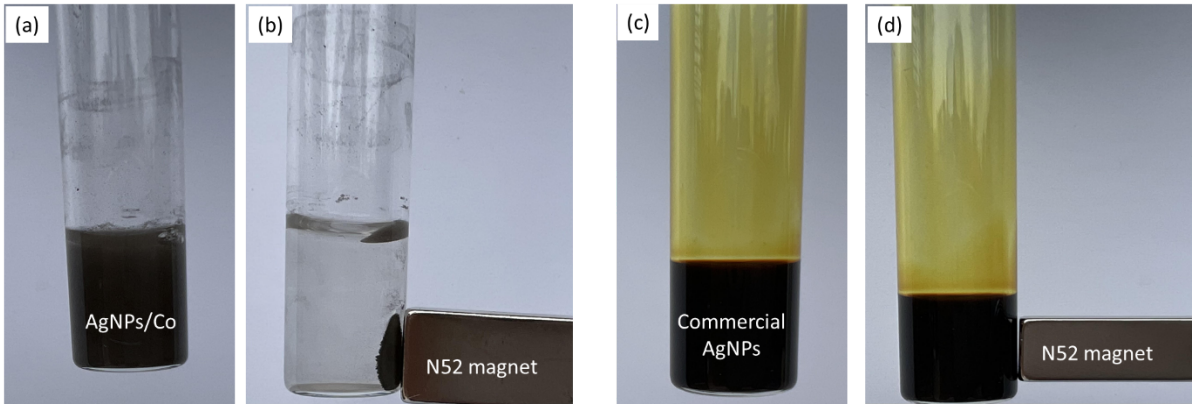


Figure 5. Comparison of Magnetic Response of (a) well dispersed AgNPs/Co (10 µg/mL) in water; (b) reaction of AgNPs/Co (10 µg/mL) to magnet; (c) well dispersed commercial silver nanoparticles (10 µg/mL) in water; (d) no reaction of commercial silver nanoparticles (10 µg/mL) to magnet.

After leaving the collected nanocomposites to dry for 24 hours, the weight of the nanocomposites remained at 0.2 g, demonstrating that there is a 100% removal rate of the AgNPs/Co with the N52 magnet. All tests were conducted in triplicate. The AgNPs/Co can be fully collected through magnets, allowing a facile and safe filtration method that prevents the risks of silver poisoning and further environmental and human health damage.

3.4. Correlation Between Reaction Time and *E. coli* Cultures

To verify the effectiveness of AgNPs/Co at different concentrations in neutralizing *E. coli* bacteria, the antibacterial rate was calculated by subtracting the total number of colonies from each sample from the total number of colonies from the control, which was then divided by the total number of colonies from the control.

The antibacterial results signify a strong correlation between the reaction time of the AgNPs/Co and their antibacterial efficiency (**Figure 6**). The antibacterial rate increases as the reaction time increases. When the AgNPs/Co react with *E. coli* for 1 minute, 94.2% of the bacteria are killed. When the AgNPs/Co react with *E. coli* for 10 minutes, the antibacterial rate increases to 99.6%. The 5.4% augmentation, as illustrated by the graph and images of the plates below, manifests as a noteworthy disparity in both antibacterial efficacy and its potential consequences on human health.

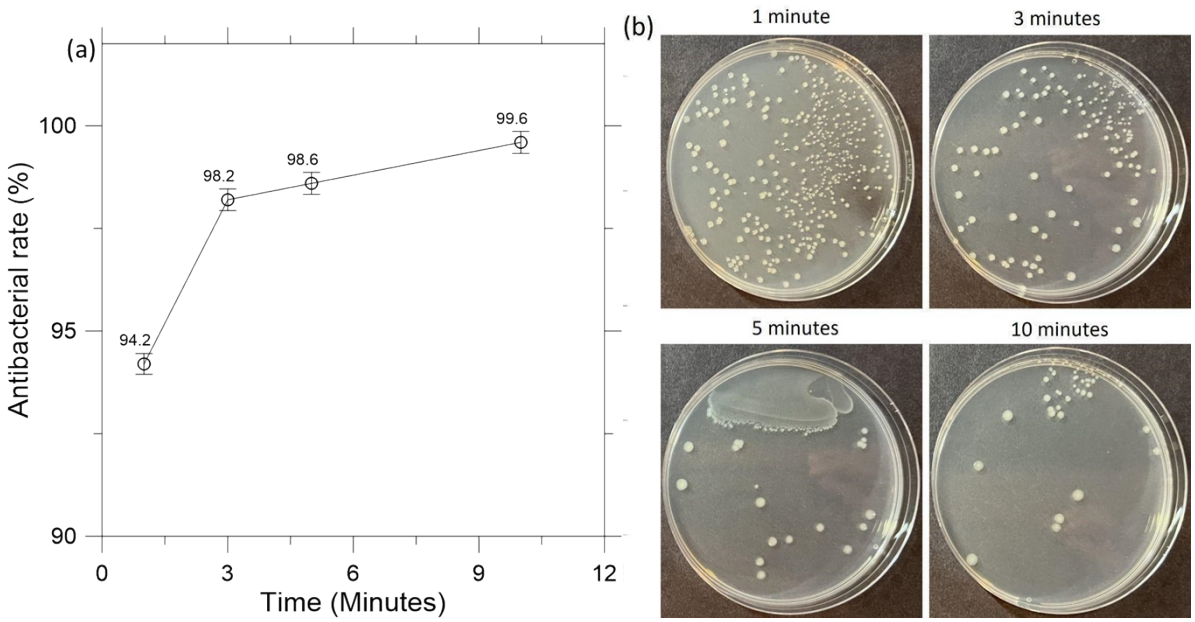


Figure 6. Antibacterial Efficiency of AgNPs/Co (a) *E. coli* antibacterial efficiency rate percentage as a function of time (minutes). (b) Images show the *E. coli* antibacterial efficiency of AgNPs/Co at 1, 3, 5, and 10 minutes.

3.5. Effects of the Material Concentration

Concentration was manipulated by decreasing the volume of the solution. The first sample, which had an AgNPs/Co concentration of 10 mg/mL, was created by combining 90 mL of DI water

and 10 mL of an *E. coli* solution that was diluted to 10^{-6}. The second sample, with an AgNPs/Co concentration of 20 mg/mL, was created by combining 45 mL of DI water and 5 mL of the same diluted *E. coli* solution. The third sample, with an AgNPs/Co concentration of 30 mg/mL, was created by combining 30 mL of DI water and 3 mL of the same diluted *E. coli* solution.

Results show that the concentration of AgNPs/Co plays a strong role in antibacterial efficiency. As the concentration increased, the number of viable *E. coli* colonies decreased. The increased concentration allows the *E. coli* to have increased interactions with the AgNPs/Co. The bacteria's outer membrane interacts with the high surface to volume ratio and small particle size of the AgNPs/Co, which causes a change in the membrane shape and permeability. As the Co-core membranes cross into the *E. coli* cell's membrane, the toxic Co and Ag ions interact with thiol-containing proteins in the cell wall, leading to reactive oxygen species reactions, DNA damage, nucleus breakdown, inhibition of enzyme biosynthesis, and an imbalanced electron transport within the cell.[21,23] These reactions can result in the *E. coli* cell membrane rupturing and ultimate cell destruction. Therefore, as time increases, based on the concentration of the *E. coli* solution, the AgNPs/Co are able to inactivate a larger number of cells, as seen in **Figures 6 and 7**.

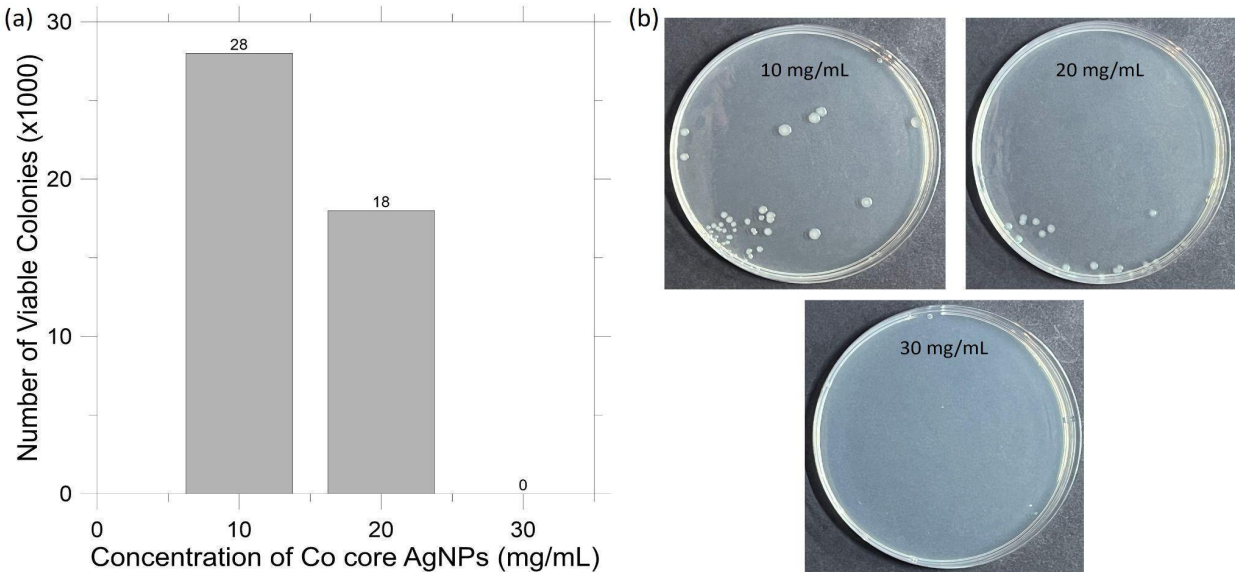


Figure 7. Antibacterial Efficiency of AgNPs/Co in different concentrations**;** (a) Number of viable colonies with a dilution factor of 10-3 with the concentration of AgNPs/Co in mg/mL. (b) Images show the number of viable colonies of AgNPs/Co for each concentration (10 mg/mL, 20 mg/mL, 30 mg/mL).

Concentrations of the silver acetate were manipulated when synthesizing the AgNPs/Co. Three different concentrations (50 mg, 100 mg, 0.200 mg) were tested for each of the three concentrations of AgNPs/Co. **Figure 8** clearly shows a correlation between the concentration of silver acetate and the antibacterial efficiency of the nanocomposites. With more added silver acetate, more silver nanocomposites would form around the cobalt core, allowing a greater surface area of the silver and a stronger presence of the silver antibacterial properties.

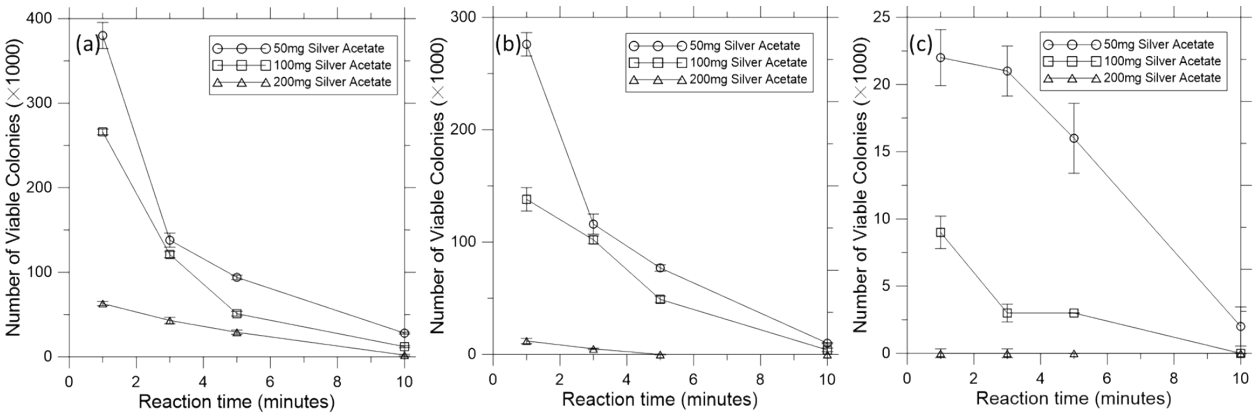


Figure 8. Different concentrations of silver acetate as a synthesis material and reaction time of AgNPs/Co in *E. coli* solution.

4. CONCLUSION

The successful synthesis of AgNPs/Co nanocomposites using the polyol process was achieved. The efficiency of these nanocomposites can be adjusted by controlling reaction time and concentration. The study evaluated the magnetic efficiency of the synthesized AgNPs/Co nanocomposite. Subsequently, the disinfection efficiency of AgNPs/Co was assessed in E. coli-contaminated water at concentrations ranging from 10 to 50 µg. The results of our research demonstrated an impressive antibacterial efficiency rate of 99.6% when utilizing the proposed AgNPs/Co nanocomposite. Moreover, the novel magnetic nanocomposites exhibited a collecting efficiency rate of 100%, indicating their effectiveness in capturing and removing the AgNPs/Co from the treated water. While further tests regarding the reusability of the nanocomposites were not conducted, it can be inferred that the AgNPs/Co can be reused due to the absence of visible nanoparticle damage, disassociation, or dissolution.

The introduction of AgNPs/Co as a disinfection strategy holds great promise for enhancing wastewater treatment processes, ensuring better removal of microbial contaminants, and reducing

the release of AgNPs/Co into the environment. Further research and development in this area may lead to the implementation of efficient and sustainable strategies for water disinfection, benefiting both human health and the ecosystem, and contribute to improved wastewater management in healthcare settings.

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