Title

Redesigning Water Disinfection Using Recyclable Nanomaterials and Metal Ions: Evaluation with Escherichia coli

Journal

ACS ES&T Water, 1(1)

ISSN

2690-0637

Authors

Gao, Qian

Keller, Arturo A

Publication Date

2021-01-08

DOI

10.1021/acsestwater.0c00066

Peer reviewed

ACS ES&T Water

pubs.acs.org/estwater

Article

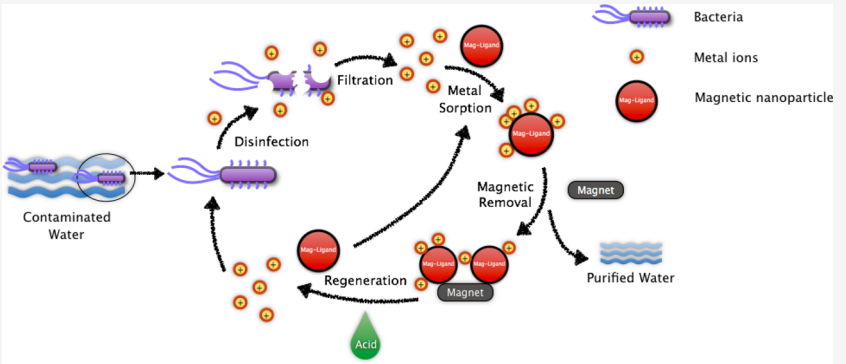
Redesigning Water Disinfection Using Recyclable Nanomaterials and Metal Ions: Evaluation with *Escherichia coli*

Qian Gao and Arturo A. Keller*

Cite This: https://dx.doi.org/10.1021/acsestwater.0c00066

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: We present a novel disinfection method that redesigns the conventional approach, by recycling the disinfectant. This can lead to lower energy requirements and minimize environmental impacts. In this study, metal ions were mixed with target microorganisms in water for disinfection, followed by the sorption of metal ions with magnetic nanoparticles for reuse. As a proof of concept, the disinfection effectiveness values of various metal ions (e.g., Ag^+, Cu^{2+}, and Zn^{2+}) on a target microorganism, *Escherichia coli* K12, were compared. Only Ag^+ exhibited a bactericidal effect on *E. coli* K12, while Cu^{2+} and Zn^{2+} just reduced the growth rate. The disinfection efficiency of Ag^+ remained stable within a range of environmental conditions (pH, temperature, water hardness, nutrient content, and initial cell concentration), indicating that Ag^+ speciation and effectiveness are not modified. The initial ratio of Ag^+ per cell is a major factor that will determine disinfection effectiveness. Sorption of Ag^+ by one type of magnetic nanomaterial (Mag-Ligand) was studied to explore the removal of Ag^+ after disinfection. Mag-Ligand can effectively decrease the concentration of Ag^+ from 100 mg/L to ~100 μg/L in one cycle, and below 10 μg/L in three recovery cycles. Changes in environmental conditions (pH, concentration of Cl^-, water hardness, and addition of *E. coli* K12) were studied to determine how these changes will affect the sorption process. The results showed that sorption capacity will be influenced when the concentration of free Ag^+ is decreased (e.g., when the Cl^- concentration is increased) or when there are competitive metal ions in the aqueous environment (i.e., water hardness). Sorption efficiency remained stable when the speciation of Ag^+ was not influenced (e.g., pH and addition of *E. coli* K12). The regeneration of Ag^+ was studied to evaluate the reuse of the disinfectant. We demonstrate that Ag^+ can be recovered after sorption in an acidic environment, and the recovery remains >80% after five continuous cycles, indicating that this disinfection method may be sustainable for practical use.

KEYWORDS: *microorganism contamination, bactericidal effectiveness, magnetic nanoparticles, disinfectant regeneration, drinking water treatment*

1. INTRODUCTION

Waterborne pathogens are one of the major sources of microbial contamination in water. The U.S. Environmental Protection Agency (EPA) has determined that >500 waterborne pathogens can increase human health risk by spreading diseases in drinking water.[1] Among various waterborne pathogens, *Escherichia coli* is a ubiquitous and widely studied bacterium. *E. coli* is a rod-shaped, Gram-negative bacterium that is commonly found in the lower intestine of warm-blooded organisms.[2] As one of the most common microbial contaminants in natural waters, *E. coli* is usually used as an indicator organism to evaluate the effectiveness of water disinfection.[3,4] Although most strains of *E. coli* are harmless, there are some pathogenic strains that can cause severe diseases (such as diarrhea) and are a major concern in public

Received: July 14, 2020
Revised: September 1, 2020
Accepted: September 16, 2020

health.[5−7] For example, an outbreak of diarrheagenic *E. coli* occurred on June 8, 2015, when a group of middle school students from Korea developed diarrhea and vomiting after attending a school camp. Further microbiological analysis of patient stools and environmental water samples indicated that the contamination of water bodies by enterohemorrhagic *E. coli*, enteropathogenic *E. coli*, and enteroaggregative *E. coli* was responsible for this outbreak.[8] Another outbreak of pathogenic *E. coli* happened in Japan in April and May 2011, when 181 patients suffered from serious food poisoning because of enterohemorrhagic *E. coli* strains O111:H8 and O157:H7 from raw beef dishes.[9] Since the outbreak of pathogenic *E. coli* may lead to severe bloody diarrhea and hemolytic uremic syndrome (HUS),[10] it is essential to develop efficient disinfection approaches, to improve water quality and protect human health.

Disinfection methods have been explored, studied, and developed ever since ancient times. Several important factors need to be considered when choosing a disinfection treatment, including water characteristics, final effluent quality, disinfectant agent toxicity, disinfection byproduct formation, local characteristics, and energy and other costs.[11] On the basis of these needs, many disinfection technologies have been explored and developed for different environmental purposes. Traditional disinfection technologies include chlorination, ozonation, and ultraviolet (UV) radiation. While traditional disinfection processes have demonstrated very good performance, the disadvantages of these conventional methods cannot be ignored. For example, chlorine may react with natural organic matter (NOM) in the source water and produce disinfection byproducts (DBPs) during the disinfection process,[12] and some DBPs have proven to be toxic and will threaten human health.[13] To overcome the issues related to production of DBPs,[14] high energy cost,[15] frequent maintenance,[16,17] and other aspects,[18,19] a number of novel disinfection methods have been explored in recent years, among which the application of nanotechnology in disinfection has generated considerable interest. In some studies, nanomaterials are embedded in water treatment membranes for disinfection and purification.[20−22] However, because the technology relies on the release of metal ions, which are left in the treated water, these nanotechnologies have a relatively short life and must be replaced frequently. The metal ion is completely lost, remains in the treated water, passes through the human body, and eventually is discharged to the environment, with a single use. Dissolution and leakage of the nanomaterials not only reduce the disinfection efficiency of the filters and membranes for future use but also threaten ecological health when these waters eventually reach the environment.[23] Separation of nanoparticles from water after disinfection is another challenge, due to their nanoscale, and they cannot be efficiently separated from water by classic water treatment.[24] In addition, the cost of synthesizing these nanocomposite filters and membranes is a major concern for practical applications.[11]

Given the issues related to microbial contamination of water sources, the need to meet more stringent requirements, and the disadvantages of current disinfection processes, we propose a radically different and sustainable disinfection method, using metal ions as the disinfectant, to be recovered through sorption after disinfection by magnetic nanoparticles coated with chelating agents, and recovered under proper conditions for reuse. The objectives of this study were (1) to investigate the disinfection effects with metal ions on target microorganisms and evaluate the influence of different environmental conditions (e.g., pH, temperature, and water hardness), (2) to study the sorption of metal ions with magnetic nanoparticles and the influence of different environmental conditions on this process, and (3) to explore the conditions needed to recover the metal ions from the magnetic nanoparticles for reuse.

2. MATERIALS AND METHODS

2.1. Materials. The *E. coli* K-12 strain was purchased from Carolina Biological Supply and used as a target microorganism in this study. Tryptic soy broth (TSB) and tryptic soy agar, silver nitrate, copper sulfate, zinc chloride, nitric acid, sulfuric acid, sodium hydroxide, sodium chloride, and calcium carbonate were purchased from Fisher Scientific. The recipe of TSB is shown in Table S1. Sodium dihydrogen phosphate was purchased from Acros Organics (Geel, Belgium). Mag-Ligand was synthesized using the method developed in our previous study,[25] and the maghemite [iron(III) oxide] nanoparticles (30 nm in diameter) used for synthesis were purchased from Alfa Aesar. In brief, the maghemite nanoparticles were dispersed in toluene and coated with amino groups by being mixed with (3-aminopropyl)triethoxysilane and refluxed in a water bath (90 °C) for 2 h. After the nanoparticles had been cooled to room temperature, ethylenediaminetetraacetic acid (EDTA) and pyridine were added to the solution to yield maghemite nanoparticles functionalized with EDTA. The pH of the solution was adjusted, and the synthesized magnetic nanoparticles were rinsed and dried at room temperature. All chemicals were used as received without further purification. All solutions were prepared with deionized water (18 MΩ cm) from a Barnstead NANOpure Diamond Water Purification System.

2.2. Disinfection of *E. coli* K12 with Ag^+, Cu^{2+}, and Zn^{2+}. A stock culture of *E. coli* K12 was prepared by inoculating the *E. coli* K12 strains in 50 mL of TSB medium and growing them at room temperature (20 °C) with constant shaking (200 rpm) overnight. Two milliliters of a stock culture was transferred into 50 mL of fresh TSB medium and inoculated for 1 h before disinfection, to make sure the growth of *E. coli* K12 was within the exponential phase. After inoculation, the culture was centrifuged at 5000 rpm for 10 min, and the supernatant was disposed. The pellet was then washed with TSB medium two or three times and then redispersed and diluted with fresh TSB medium until the optical density at 600 nm (OD_{600}) of the diluted sample reached 0.05. The OD_{600} values of bacterial samples were determined by a UV-1800 ultraviolet−visible spectrophotometer (Shimadzu Scientific Instruments Inc.).

The disinfection of *E. coli* K12 was performed in 24-well tissue culture plates. Silver nitrate, copper sulfate, and zinc chloride were utilized as disinfectants. An *E. coli* K12 solution with an OD_{600} of ∼0.045 was exposed to Ag^+, Cu^{2+}, and Zn^{2+} at different concentrations (5−100 mg/L for Ag^+ and 5−500 mg/L for Cu^{2+} and Zn^{2+}) for different contact times (≤10 h) in TSB medium. The OD_{600} of each sample was measured after exposure.

Different operating conditions for disinfection were tested to explore their influence on disinfection. Temperature, pH, water hardness, and nutrient content are typical aqueous environmental conditions that need to be considered. Thus, each of the conditions was changed within a proper range. The pH of the TSB medium was adjusted from 6 to 8 using 0.1 M nitric

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX

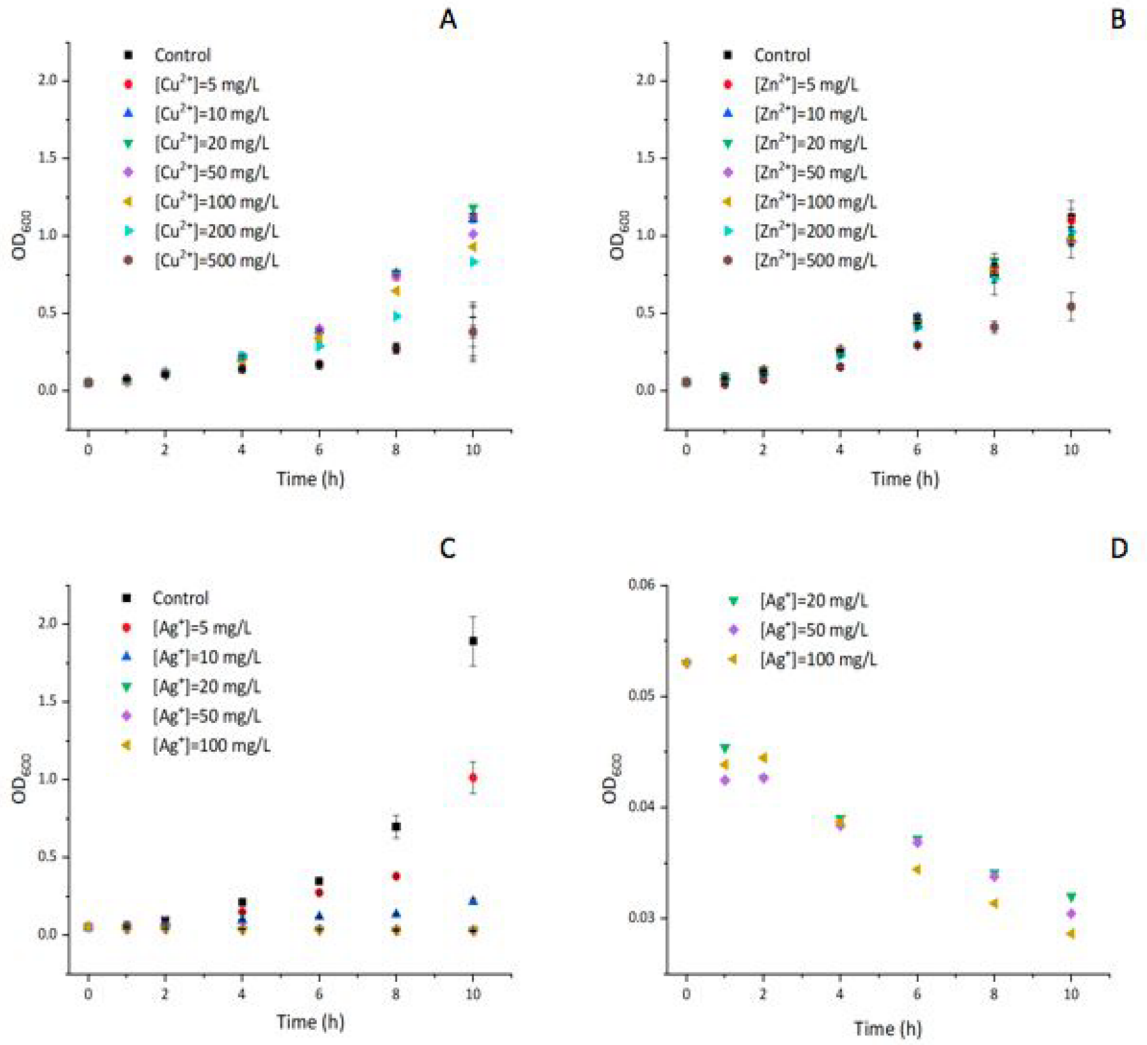


Figure 1. Effect of disinfection on *E. coli* K12 (initial OD_{600} = 0.053) with (A) Cu^{2+} (5−500 mg/L), (B) Zn^{2+} (5−500 mg/L), (C) Ag^{+} (5−100 mg/L), and (D) Ag^{+} (20−100 mg/L), to show more detail for this range of concentrations.

acid and 0.1 M sodium hydroxide, and the temperature was adjusted from 10 to 30 °C. $CaCO_3$ was used to adjust the water hardness within a range from 50 to 200 mg/L, to simulate conditions from soft to moderately hard, hard, and very hard. The disinfection process was performed at different nutrient content levels by diluting the TSB medium with deionized water to different percentages (full strengths of 100%, 60%, and 10%) to determine how this factor will influence disinfection effectiveness. In addition, the initial OD_{600} of *E. coli* K12 was adjusted to different levels (0.013, 0.065, and 0.13), as well, to explore the differences in disinfection effectiveness.

2.3. Batch Sorption of Ag^{+} with Mag-Ligand. Adsorption of Ag^{+} with Mag-Ligand was evaluated under different conditions. Different amounts of Mag-Ligand particles (50.0−200.0 mg) were mixed with 20 mL of a Ag^{+} solution (100 mg/L) in 20 mL glass vials, and the vials were placed in an end-over-end shaker on a Dayton-6Z412A Parallel Shaft roller mixer with a speed of 70 rpm at room temperature (22−25 °C) for 24 h to ensure sufficient equilibration time. Studies of adsorption kinetics were carried out with the mixture of 20.0 mg of Mag-Ligand and 20 mL of a Ag^{+} solution (100 mg/L) under the same conditions but for a set amount of time, varying from 30 min to 24 h. All of the studies were performed at pH 7 and room temperature. After being mixed well, the Mag-Ligand particles were separated from the aqueous phase with an Eclipse magnet. Samples were collected from the supernatant and diluted with 2% HNO_3. Then the concentration of Ag^{+} in the samples was analyzed with an Agilent 7900 (Agilent Technologies) inductively coupled plasma mass spectrometer.

The influence of different environmental conditions on the efficiency of removal of Ag^{+} by Mag-Ligand, including pH, Cl^{-} concentration, water hardness, and the presence of bacterial cells, was studied, as well. The pH was adjusted to the desired condition (from 6 to 8) by using sodium dihydrogen phosphate buffer. Different concentrations of Cl^{-} (1−100 mg/L) were added to the mixture to explore the possible influence on sorption, as different combinations of Cl^{-} and Ag^{+} may affect the sorption behavior of Mag-Ligand. $CaCO_3$ over a range of concentrations from 50 to 200 mg/L was used to adjust water hardness. In other experiments, different concentrations of bacteria [10^{2}−10^{6} colony-forming units (CFU)/mL of *E. coli* K12] were introduced into the sorption system, to determine if the presence of bacteria would influence the sorption process.

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX

2.4. Regeneration and Reuse of Ag^+. To investigate the regeneration and reuse of Ag^+ after sorption with Mag-Ligand, 100 mg/L Ag^+ was adsorbed onto the Mag-Ligand particles, followed by separation of Mag-Ligand from solution with the hand-held magnet. The Mag-Ligand collected was then washed with 0.01 M H_2SO_4 (pH 1.70) for 30 min at room temperature, and the Ag^+ concentration in the supernatant solution was determined by inductively coupled plasma mass spectrometry. The acid-washed Mag-Ligand particles were then reused for subsequent Ag^+ sorption experiments, and the sorption, extraction, and reuse processes were repeated five times.

2.5. Data Analysis. All tests in this study were performed in triplicate, and analysis of variance (ANOVA) was used to test the significance of results. A p values of <0.05 was considered to be statistically significant. The p values of each test are listed in Table S2.

3. RESULTS AND DISCUSSION

3.1. Disinfection of *E. coli* K12 with Metal Ions. *3.1.1. Comparison of the Disinfection Effect with Various Metal Ions.* The results of disinfection with Ag^+, Cu^{2+}, and Zn^{2+} at different concentrations and contact times are shown in Figure 1. Generally, the effectiveness of a disinfectant on its target can be described as bacteriostatic or bactericidal. Bacteriostatic refers to the phenomenon in which bacteria are inhibited partially or totally from reproducing by the addition of disinfectant, and bactericidal means killing the bacteria.[26] In this study, the effectiveness of disinfection was determined by comparing the OD_{600} of samples exposed to disinfectants with that of the control at the same contact time. Measurement of OD_{600} is a common technique for estimating the concentration of bacteria in microbiology, as the OD_{600} is quantitatively related to the biomass in cell suspension within a certain range.[27] Although OD_{600} cannot provide quantitative information about the living cells in samples, it can serve to determine whether the growth of bacteria is influenced by the disinfectants. An increase in OD_{600} correlates linearly with an increase in the number of living cells within the experimental range, while a decrease in OD_{600} indicates there are fewer cells, likely since cell death occurred. The results of disinfection of *E. coli* K12 showed that the effectiveness is related to both the type and concentration of metal ions. As shown in Figure 1, addition of Cu^{2+} and Zn^{2+} slowed the growth rate of *E. coli* K12 only compared to that in the control group. However, no bacteriostatic or bactericidal effect was found at any concentration (5−500 mg/L in this study), as the OD_{600} increased continuously at longer contact times. Compared to Cu^{2+} and Zn^{2+}, Ag^+ had a much better disinfection efficacy. Cu^{2+} has a stronger disinfection ability on *E. coli* K12 than Zn^{2+}, as the growth rate of *E. coli* K12 exposed to Zn^{2+} was closer to the control than that exposed to Cu^{2+}. As shown in Figure 1D, the bactericidal effect appeared when the concentration of Ag^+ was >20 mg/L, and the effect was more noticeable at a higher concentration of Ag^+. Thus, the disinfection efficacy for these metal ions decreases in the following order: $Ag^+ \gg Cu^{2+} > Zn^{2+}$. The reason for the difference in disinfection ability among Ag^+, Cu^{2+}, and Zn^{2+} is mainly due to the mechanisms of interaction with cells.[28] Ag^+ has several ways to interfere with the metabolism of cells and destroy the cell structure by binding with membranes, enzymes, nucleic acids, and other cellular components. Compared to Ag^+, Cu^{2+} is not so effective in inactivating cells as the main mechanism is just increasing intracellular reactive oxygen species and impairing cell membranes. Zn^{2+} has the weakest ability to inactivate cells compared with Ag^+ and Cu^{2+}, as Zn^{2+} can deplete only the total cellular thiols to achieve protein dysfunction.

To further study the disinfection ability of Ag^+, we measured the survival kinetics of *E. coli* K12 exposed to a high concentration of Ag^+ (100 mg/L) and with a long contact time (≤32 h). Samples were collected and measured every 4 h (Figure 2). OD_{600} decreased until a contact time of 16 h,

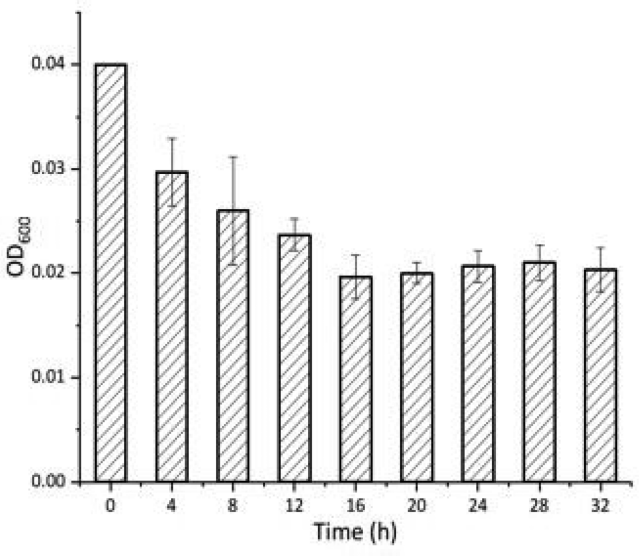


Figure 2. Disinfection kinetics with 100% nutrient and 100 mg/L Ag^+ (initial OD_{600} = 0.04).

indicating that cells were killed by Ag^+ during this time, and the rate of death of *E. coli* K12 increased with contact time. However, after contact for 16 h, the OD_{600} remained at a stable level (around 0.020) with a slight variance. To confirm if the cells were still alive after 16 h of contact time, an aliquot (0.1 mL) of the sample was transferred onto a tryptic soy agar plate for plate counting. The tryptic soy agar plate was then cultured on the incubator with a shaking speed of 200 rpm at 20 °C for >48 h, until colonies formed on the agar plate. The results of the plate counting test are shown in Figure S1. On the basis of plate counting, the concentration of living cells after treatment for 16 h with 100 mg/L Ag^+ decreased to 170 CFU/mL, slightly above the EPA standard (<126 CFU/100 mL).[29] This may be due to precipitation of Ag^+ over time, which reduces the availability of the disinfectant.

3.1.2. Influence of Different Environmental Conditions on Disinfection. 3.1.2.1. Initial Cell Concentration. Different initial concentrations of *E. coli* K12 (initial OD_{600} values of 0.013, 0.065, and 0.13) were studied, at 100 mg/L Ag^+ with a 4 h contact time, to explore their effect on disinfection effectiveness. As shown in Figure 3, the effectiveness of disinfection on *E. coli* K12 is influenced by the initial cell concentration. At the same Ag^+ dose, a lower initial OD_{600} (0.013) resulted in a larger decrease in the final OD_{600} (~34% of the initial OD_{600}) compared to the higher initial OD_{600} (0.13) where the final OD_{600} was around 76% of the initial OD_{600}. In all treatment cases, the final OD_{600} was less than the initial OD_{600}, which was not the case for the control. Note that even dead cells contribute to OD_{600}, but as shown above, the

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX

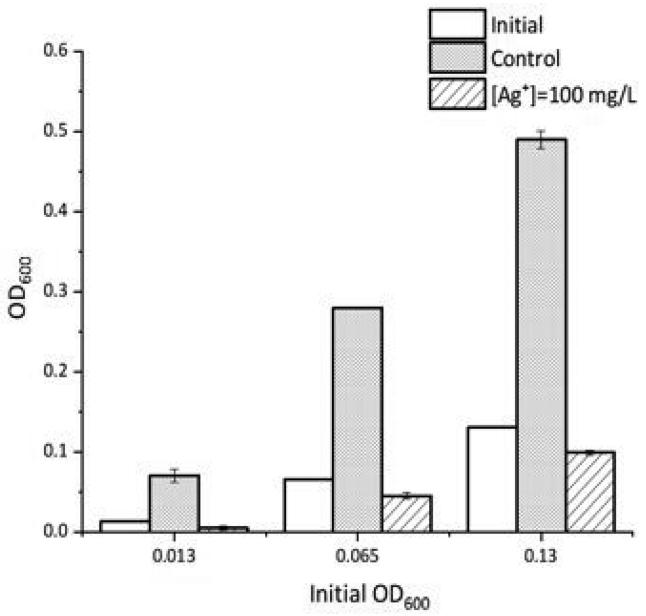


Figure 3. Comparison of disinfection effectiveness at 100 mg/L Ag^+ with a 4 h contact time and different initial OD_{600} values.

CFU can be quite low after treatment. This is supported by previous research stating that the disinfection effect is determined by the initial level of silver per cell,[30] and a higher initial level of silver will lead to a more rapid disinfection process.

3.1.2.2. pH. The effectiveness of disinfection at 100 mg/L Ag^+ with a 4 h contact time and an initial OD_{600} of 0.043 at different pH values (6, 7, and 8) was evaluated. As shown in Figure 4A, after 4 h the increase in OD_{600} in the control group was a function of the initial pH. A more acidic environment (pH 6) decreased the growth rate of *E. coli* K12, compared to that under alkaline or neutral conditions. In the group exposed to Ag^+, there was a decrease in OD_{600} (from 0.043 to 0.029 at pH 6, from 0.043 to 0.028 at pH 7, and from 0.043 to 0.026 at pH 8) after the treatment. However, the differences in the final OD_{600} between treatments were not significant ($p = 0.057 > 0.05$). Ag^+ is a stable species in the environment from pH 6 to 8,[31] leading to a stable disinfection effectiveness for *E. coli* K12.

3.1.2.3. Water Hardness. Water hardness, usually expressed as the concentration of $CaCO_3$ in water, is one of the main environmental factors that determine the quality of freshwater. In this study, different concentrations of $CaCO_3$ (50, 100, and 200 mg/L) were added to simulate different degrees of water hardness. As shown in Figure 4B, the addition of $CaCO_3$ slightly influences the final OD_{600} in the control. However, no influence on the disinfection effectiveness was observed under different water hardnesses ($p = 0.064 > 0.05$), as the addition of $CaCO_3$ did not change the speciation or concentration of Ag^+. This confirms the results of a previous study of the disinfection effectiveness of Ag^+ even when the water is very hard.[32] Thus, water hardness will not influence the disinfection process.

3.1.2.4. Temperature. Different temperatures (10, 20, and 30 °C) were considered to explore their influence on disinfection effectiveness. As shown in Figure 4C, temperature is a factor that will affect the growth of *E. coli* K12. At a low temperature (10 °C), the final OD_{600} of the control is lower than the initial OD_{600}, indicating that a cold environment will inhibit the growth of *E. coli* K12. At a higher temperature (20 or 30 °C), the final OD_{600} of *E. coli* K12 in the control increased, and growth was accelerated by an increase in temperature, while the temperature is below optimal (37 °C for *E. coli*).[33] On the basis of the result that disinfection efficacy decreases with a higher cell concentration (Figure 3), it is reasonable that the efficacy is lower at a higher temperature than that at a lower temperature (Figure 4D).

3.1.2.5. Nutrient Content. Nutrient content is another environmental factor that may affect the disinfection process, as it influences the degree to which bacteria grow in the aqueous environment. Some organic matter in water may be utilized by bacteria as nutrients and result in an outbreak. In this study, TSB medium with organic matter such as tryptone and glucose is used for the culture of *E. coli* K12, to provide an environment with abundant nutrients. However, the nutrient content in water samples from natural environments is usually not as rich as that under laboratory conditions. Thus, a batch of experiments with different concentrations of medium were performed to explore the possible influence of organic matter as nutrients. The TSB medium (full strength of 100%) was diluted using deionized water to different degrees (60% and 10% of original) for the study, and the experiment was performed with 100 mg/L Ag^+, an initial OD_{600} of 0.062, and a ≤32 h contact time. As shown in Figure 5A, which is the disinfection effectiveness with a 24 h contact time, the change in nutrient content did influence the growth rate of *E. coli* K12 in both unexposed and exposed cells, but in quite different ways. In the unexposed group (control), the final OD_{600} in different nutrient conditions follows the order 100% nutrient > 60% nutrient > 10% nutrient, indicating that the nutrient conditions and growth of *E. coli* K12 have a positive correlation; more nutrients, more growth. However, in the group exposed to 100 mg/L Ag^+ (Figure 5B), the final OD_{600} and different nutrient conditions followed the order 10% nutrient > 60% nutrient > 100% nutrient. A possible explanation may be that a low-nutrient environment stimulates the metabolism of bacteria,[34] making it more difficult to destroy the cell structure and thus reducing the effectiveness of disinfection.

The contact time was extended to determine if the OD_{600} would remain stable after enough contact time (≤32 h) with 100 mg/L Ag^+ under 10%, 60%, and 100% nutrient conditions (Figure 5C). The time needed for the OD_{600} to reach a stable level in various treatments was different. In the treatment with 10% nutrients, the OD_{600} decreased by 22.6% in 24 h and remained stable at the low level ($OD_{600} = 0.048$; $p = 0.198 > 0.05$). In the treatment with 60% nutrients, it took 24 h for the OD_{600} to reach a stable level of 0.047 ($p = 0.332 > 0.05$) with a decrease of 24.2% compared to the initial OD_{600}. In the treatment with 100% nutrients, the decrease in the OD_{600} at an early stage (<16 h) was more significant (27.4%) than for nutrient-poor conditions, and the OD_{600} remained at 0.045 after 16 h ($p = 0.211 > 0.05$). Thus, disinfection effectiveness reaches a stable level faster in a nutrient-rich environment than under nutrient-poor conditions.

3.2. Sorption of Metal Ions with Mag-Ligand.

3.2.1. Sorption Isotherm and Kinetics. The sorption of Ag^+ (100 mg/L) at different concentrations of Mag-Ligand (2.5–10 g/L) at pH 7 and room temperature was studied. As shown in Figure 6A, the removal efficiency of Ag^+ increased substantially with a larger adsorbent dose of ≤5 g/L, reaching 96%. Above 5 g/L, the removal efficiency increased asymptoti-

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX

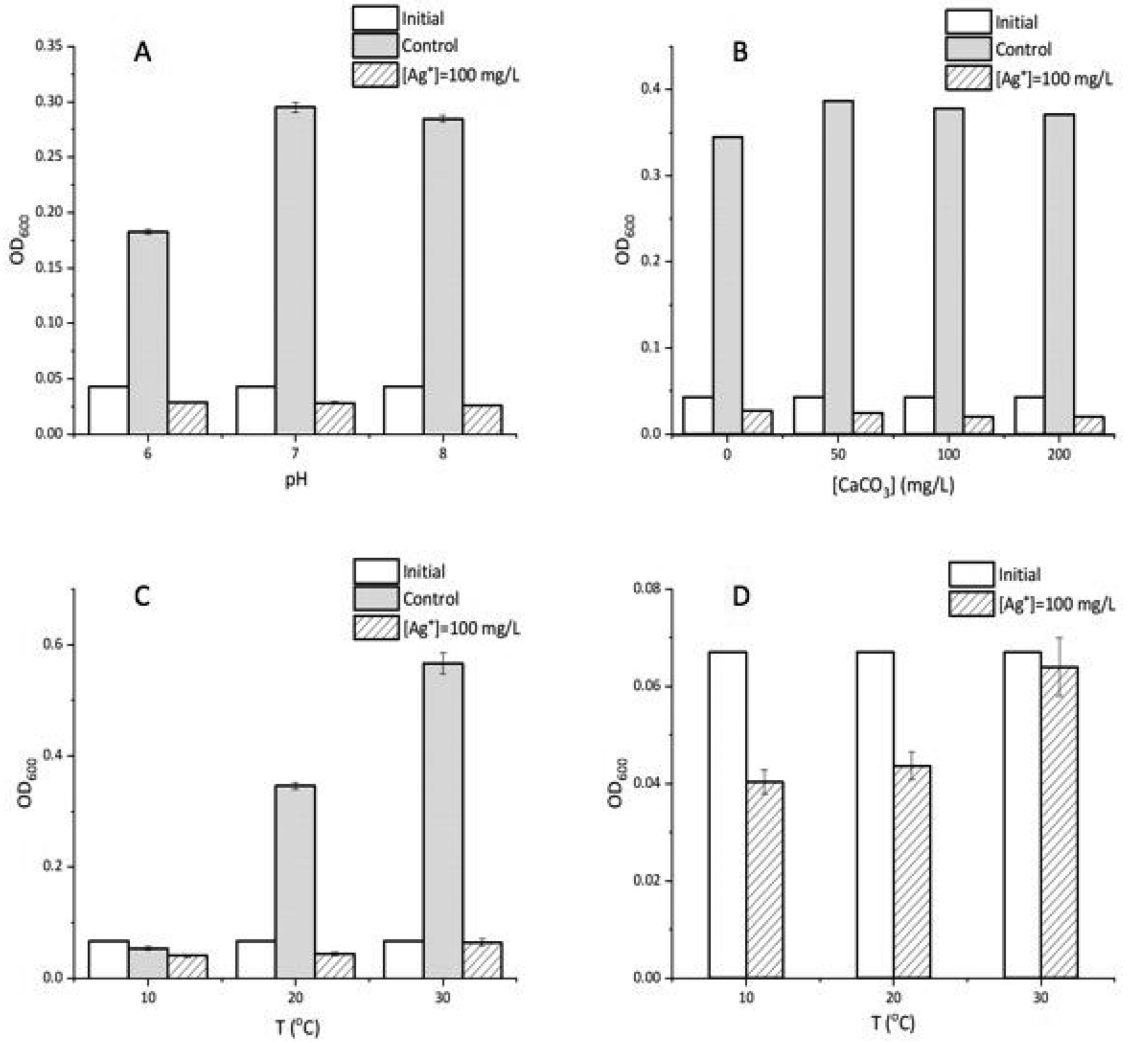


Figure 4. Disinfection effectiveness as a function of (A) pH, (B) water hardness, and (C) temperature with 100 mg/L Ag^+ and a 4 h contact time (initial OD_{600} values of 0.043, 0.043, and 0.067, respectively). (D) OD_{600} for the Ag^+ treatment condition at different temperatures.

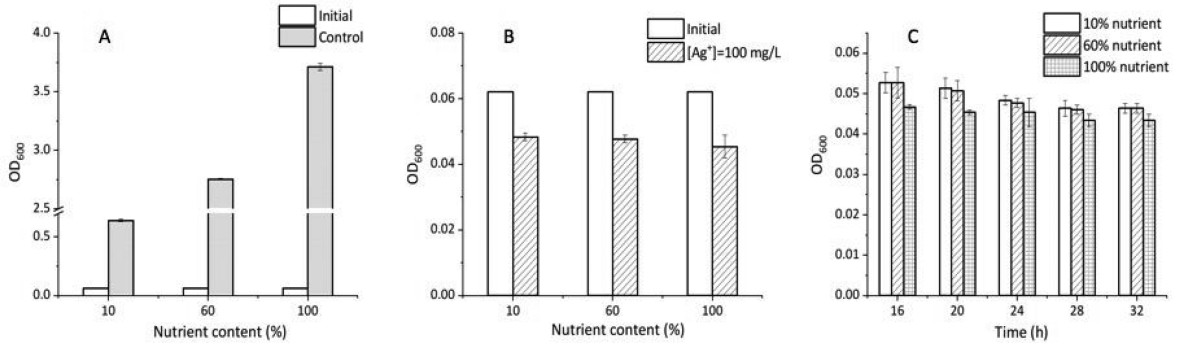


Figure 5. Disinfection tests under various nutrient contents with 100 mg/L Ag^+, an initial OD_{600} of 0.062, and a 24 h contact time. (A) Influence of nutrient content on growth rate in the control. (B) Influence of nutrient content on disinfection effectiveness. (C) Comparison of survival kinetics with different nutrient conditions (10%, 60%, and 100%).

cally to 98.3%, probably due to the challenge of keeping the Mag-Ligand particles suspended at concentrations of >5 g/L.

The kinetics of sorption of 100 mg/L Ag^+ with Mag-Ligand (2.5 g/L) were studied at pH 7 and room temperature (Figure 6B). Rapid sorption occurred, with the removal efficiency reaching a maximum of 55% at this dosage within 3 h. The results are consistent with our previous study of Mag-Ligand kinetics with other metal ions.[25] The kinetics is influenced by the amount of EDTA coated on Mag-Ligand, as well as the affinity between metal ions and EDTA.[35]

As discussed above, the removal efficiency reaches an asymptotic level of 98.3% even with an increasing Mag-Ligand dose. However, the residual concentration of Ag^+ is above the EPA's secondary maximum contaminant standard (100 μg/

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX

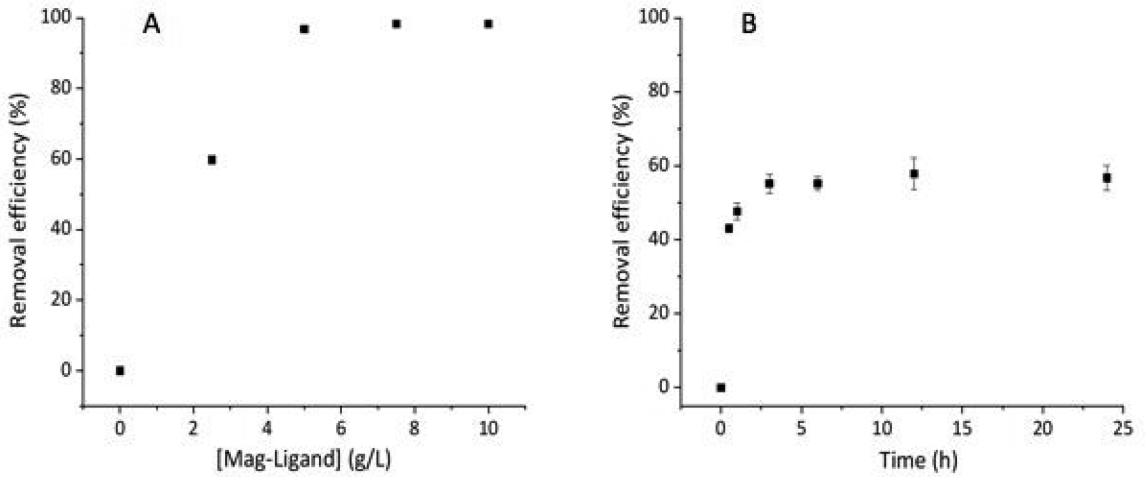


Figure 6. (A) Adsorption of Ag^+ on Mag-Ligand as a function of adsorbent dose with 100 mg/L Ag^+. (B) Ag^+ sorption uptake vs time (with 100 mg/L Ag^+ and 2.5 g/L Mag-Ligand).

L).[36] To decrease the concentration of Ag^+ below 100 μg/L, another experiment was performed, with the same total dose of Mag-Ligand but applied in separate sorption cycles. The total dose of Mag-Ligand was 10 g/L, and the initial Ag^+ concentration was 100 mg/L. Three different sorption experiments were evaluated: (1) 10 g/L in cycle 1, (2) 7.5 g/L in cycle 1 and 2.5 g/L in cycle 2, and (3) 5 g/L in cycle 1, 2.5 g/L in cycle 2, and 2.5 g/L in cycle 3. In experiments 2 and 3, after sorption had been allowed to reach equilibrium (24 h), Mag-Ligand was separated from the suspension using a magnet, and immediately thereafter, additional Mag-Ligand was dosed as indicated above. Samples were collected after each cycle, and the concentration of Ag^+ was measured. As indicated in Table 1, the concentration of Ag^+ decreased to 7 μg/L after three sorption cycles. Compared to the sorption with just one cycle, the removal effectiveness was substantially improved without increasing the total Mag-Ligand dose. Further study showed that the concentration of Ag^+ can be decreased to below the secondary MCL after three sorption cycles with a shorter time [2 h for each cycle (Table S3)]. Thus, increasing the number of sorption cycles can decrease the Ag^+ concentration well below the secondary MCL; further optimization could be done to determine the minimum total Mag-Ligand dose and sorption time, depending on conditions.

Table 1. Concentrations of Ag^+ after Different Continuous Sorption Cycles

	[Ag^+] after sorption (mg/L)		
Mag-Ligand dose	cycle 1	cycle 2	cycle 3
10 g/L in cycle 1	1.66		
7.5 g/L in cycle 1 and 2.5 g/L in cycle 2	1.67	0.11	
5 g/L in cycle 1, 2.5 g/L in cycle 2, and 2.5 g/L in cycle 3	3.11	0.14	0.007

3.2.2. Influence of Different Environmental Conditions on Adsorption. 3.2.2.1. pH. The influence of pH on the adsorption process of Ag^+ with Mag-Ligand was evaluated with 100 mg/L Ag^+ and 5 g/L Mag-Ligand (Figure 7). Removal efficiency remained stable at around 96−97% when the pH varied from 6 to 8 ($p = 0.179 > 0.05$) (Figure 7A). This is mainly due to the sorption mechanism of Ag^+ and EDTA. The complex formation constant, $K_{Ag\text{-}EDTA}$, of Ag^+ and EDTA is very high with a $\log(K_{Ag\text{-}EDTA})$ of 7.20,[35] indicating that Ag^+ forms a stable complex with the EDTA coated on the surface of Mag-Ligand, resulting in a high removal efficiency. Because the change in pH in the range of 6−8 does not affect the aqueous speciation of Ag^+,[37] the removal efficiency is not influenced.

3.2.2.2. Cl^-. Different concentrations of Cl^- (1, 10, and 100 mg/L) were considered to explore the influence on the adsorption process, as the presence of Cl^- could affect the speciation of Ag^+ by forming AgCl precipitates or an $AgCl_2^-$ complex in water, thus affecting the removal via Mag-Ligand. The addition of Cl^- decreased the rate of removal of Ag^+ via adsorption (Figure 7B). At a low Cl^- level (1 mg/L), a small percentage of Ag^+ (1.56% of initial Ag^+) will combine with Cl^- to form AgCl precipitates. This is calculated using the solubility product constant, K_{sp}, of AgCl, which is 1.77×10^{-10} at room temperature.[38] After sorption, 93.94% of the initial Ag^+ ions were captured by Mag-Ligand. When the concentration of Cl^- was increased to 10 mg/L, the percentage of Ag^+ in AgCl(s) increased to 27.63%, and the percentage adsorbed on Mag-Ligand decreased to 61.01%. Although AgCl(s) could be removed in the slurry in a commercial operation, the presence of Cl^- would result in a small loss of Ag^+ for reuse directly. It may be possible to redissolve Ag^+ from the slurry for reuse; this was not evaluated here. At a Cl^- concentration of 100 mg/L, soluble $AgCl_2^-$ ($\log K_{AgCl_2^-} = 5.25$) may form.[39] The Cl^- in water will compete with EDTA coated on nanoparticles to form a complex with Ag^+, resulting in a decrease in the removal efficiency to 55.36%.

3.2.2.3. Water Hardness. The addition of 50 mg/L $CaCO_3$ reduced the removal efficiency of Ag^+ by ~3.75%, compared to that with no $CaCO_3$ (Figure 7C). When the $CaCO_3$ concentration increased to 100 mg/L, the removal efficiency decreased to 89.1%. This is due to the increased concentration of dissolved Ca^{2+} in solution, which can combine with EDTA and form a complex, with a complex formation constant ($\log K_{Ca\text{-}EDTA} = 10.69$) that is higher than that of the Ag-EDTA complex.[35] However, due to the low solubility of $CaCO_3$, the dissolved Ca^{2+} is limited. At a $CaCO_3$ concentration of 200 mg/L, the removal efficiency was almost the same as at 100 mg/L $CaCO_3$ ($p = 0.123 > 0.05$).

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX

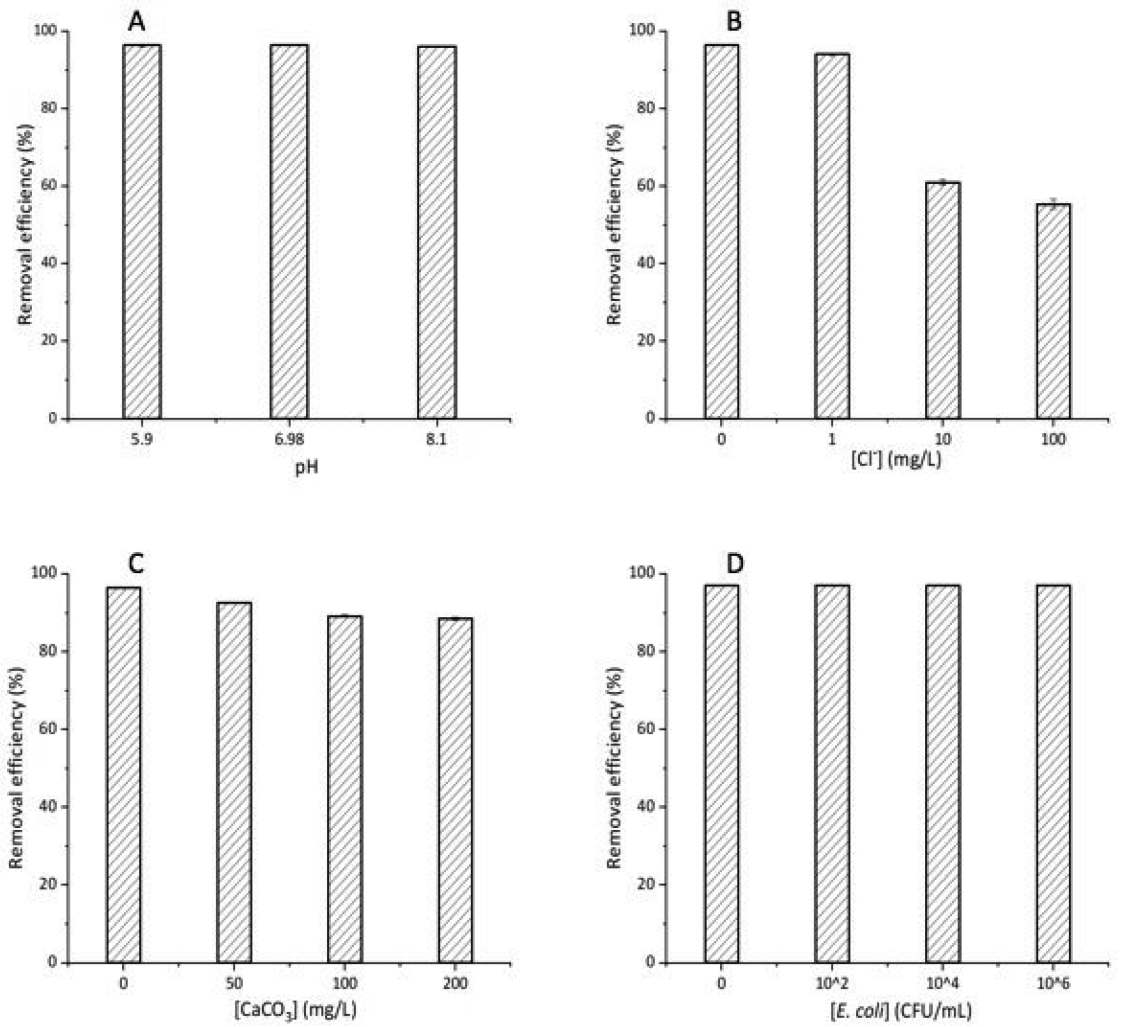


Figure 7. Influence on sorption for 100 mg/L Ag^+ and 5 g/L Mag-Ligand of different (A) pH values, (B) Cl^- concentrations, (C) water hardness values, and (D) *E. coli* concentrations.

3.2.2.4. E. coli Concentration. Sorption experiments with Ag^+ on Mag-Ligand at different concentrations of *E. coli* K12 were performed to determine if the presence of bacteria interferes with the sorption process. No significant difference was found with addition of *E. coli* K12 at different concentrations (p = 0.873 > 0.05) (Figure 7D). Ag^+ likely does not adhere to *E. coli* K12 cell walls, and *E. coli* K12 does not appear to interact with the surface of Mag-Ligand. Given this result, the removal of Ag^+ with Mag-Ligand can take place immediately after the disinfection reaches the desired level without separating the bacteria first, thus reducing the time and cost.

3.3. Regeneration and Reuse of Metal Ions and Mag-Ligand. The recovery of Ag^+ adsorbed onto Mag-Ligand was performed under acidic conditions (0.01 M H_2SO_4, pH 1.7), using the initial Ag^+ and Mag-Ligand concentrations. The recovery efficiency of Ag^+ after five sequential cycles of Mag-Ligand regeneration remained >80% (Figure 8), indicating that a large fraction of the initial Ag^+ can be reused for several cycles. Given the high price of $AgNO_3$ (e.g., $5.44/g from Sigma-Aldrich, in small quantities), recycling Ag^+ as a disinfectant will reduce the cost of this disinfection method, making it more sustainable than approaches using nanoAg in which the Ag^+ is lost after just one cycle. Mag-Ligand can be

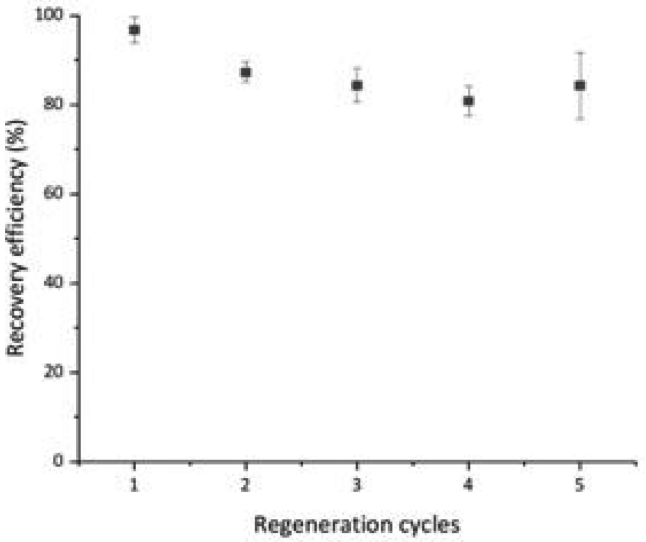


Figure 8. Recovery efficiency of Ag^+ after five Mag-Ligand regeneration cycles.

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX

captured and separated from the aqueous phase by an external magnet after use, thus reducing the potential environmental influence.

4. CONCLUSIONS

This study evaluated the disinfection effectiveness of various metal ions (Ag^+, Cu^{2+}, and Zn^{2+}) on the target microorganism, *E. coli* K12, and explored the influence of different environmental conditions on the disinfection process. To make the disinfection process more sustainable and economical, the metal ions were recovered using a magnetic nanomaterial (Mag-Ligand), which was then regenerated, recycling both the metal ion disinfectant and the nanomaterial. Ag^+ was shown to have a better disinfection efficiency than Cu^{2+} and Zn^{2+}, as the bactericidal effect occurred with only Ag^+. Factors that may determine the effectiveness of disinfection by Ag^+ include the initial level of Ag^+ per cell, as well as the speciation of Ag^+ in the environment. Different initial cell concentrations and operating temperatures will influence the disinfection effectiveness, with higher cell:Ag^+ ratios and higher temperatures decreasing disinfection effectiveness. However, the pH, water hardness, and nutrient content have minimal influence on the disinfection process, because the speciation of Ag^+ remains stable under these conditions.

The adsorption of Ag^+ by Mag-Ligand can be used to decrease the concentration of Ag^+ to <100 μg/L under the proper conditions but may require optimization of the dose of Mag-Ligand and the number of sorption cycles, depending on conditions. The mechanism of sorption of Ag^+ by Mag-Ligand is the formation of a stable complex between Ag^+ and the EDTA coated on the surface of Mag-Ligand. The influence of several environmental conditions on sorption efficiency was evaluated, as well. The addition of Cl^- will influence the concentration of free Ag^+ in solution, thus decreasing the Ag^+ removal efficiency. Water hardness decreases Ag^+ sorption, because the dissolved Ca^{2+} will compete with Ag^+ for the sorption site. The effect of hardness increases the concentration to 100 mg/L $CaCO_3$; above that level, there is no further effect of hardness on sorption. The pH has no influence on the removal efficiency, as the speciation of Ag^+ remains stable within the range in this study. Addition of *E. coli* K12 also did not affect the removal efficiency, which means that removal of Ag^+ can proceed directly after disinfection. Mag-Ligand can be regenerated under an acidic environment after sorption, and the recovery of Ag^+ remains above 80% after five sequential cycles. This novel approach allows for a recyclable disinfectant, aided by a regeneratable nanomaterial. Due to the low cost and energy requirement for synthesizing Mag-Ligand ($3.17/g under laboratory conditions), as well as the recyclable use of both the metal ions and Mag-Ligand, this method is very promising for practical use in the future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.0c00066.

Evaluation of live cells after disinfection, recipe of tryptic soy broth, statistical analysis of results, and determination of sorption time for Ag^+ removal using Mag-Ligand (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Arturo A. Keller − *Bren School of Environmental Science and Management, University of California at Santa Barbara, Santa Barbara, California 93106, United States;* orcid.org/0000-0002-7638-662X; Email: keller@bren.ucsb.edu

Author

Qian Gao − *Bren School of Environmental Science and Management, University of California at Santa Barbara, Santa Barbara, California 93106, United States;* orcid.org/0000-0002-0485-1596

Complete contact information is available at: https://pubs.acs.org/10.1021/acsestwater.0c00066

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (NSF) and the U.S. Environmental Protection Agency (EPA) under Cooperative Agreement NSF-EF0830117, as well as a UCSB Mellichamp Sustainability Fellowship. A.A.K. also appreciates Agilent Technologies for their Agilent Thought Leader Award. All photos in the manuscript (i.e., Figure S1) were taken by Q.G.

■ REFERENCES

(1) Ashbolt, N. J. Microbial contamination of drinking water and human health from community water systems. *Curr. Environ. Health Rep.* **2015**, *2* (1), 95−106.

(2) Tenaillon, O.; Skurnik, D.; Picard, B.; Denamur, E. The population genetics of commensal Escherichia coli. *Nat. Rev. Microbiol.* **2010**, *8* (3), 207−217.

(3) Li, Y.; Yang, M.; Zhang, X.; Jiang, J.; Liu, J.; Yau, C. F.; Graham, N. J.; Li, X. Two-step chlorination: a new approach to disinfection of a primary sewage effluent. *Water Res.* **2017**, *108*, 339−347.

(4) Ragazzo, P.; Chiucchini, N.; Piccolo, V.; Spadolini, M.; Carrer, S.; Zanon, F.; Gehr, R. Wastewater Disinfection: Long-Term Laboratory and Full-Scale Studies on Performic Acid in Comparison with Peracetic Acid and Chlorine. *Water Res.* **2020**, *184*, 116169.

(5) Jones, I. G.; Roworth, M. An outbreak of Escherichia coli 0157 and campylobacteriosisassociated with contamination of a drinking water supply. *Public Health* **1996**, *110* (5), 277−282.

(6) Hrudey, S. E.; Payment, P.; Huck, P. M.; Gillham, R. W.; Hrudey, E. J. A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. *Water Sci. Technol.* **2003**, *47* (3), 7−14.

(7) Lienemann, T.; Pitkänen, T.; Antikainen, J.; Mölsä, E.; Miettinen, I.; Haukka, K.; Vaara, M.; Siitonen, A. Shiga Toxin-Producing Escherichia coli O100: H−: stx 2e in Drinking Water Contaminated by Waste Water in Finland. *Curr. Microbiol.* **2011**, *62* (4), 1239−1244.

(8) Park, J.; Kim, J. S.; Kim, S.; Shin, E.; Oh, K. H.; Kim, Y.; Kim, C. H.; Hwang, M. A.; Jin, C. M.; Na, K.; Lee, J.; et al. A waterborne outbreak of multiple diarrheagenic Escherichia coli infections associated with drinking water at a school camp. *Int. J. Infect. Dis.* **2018**, *66*, 45−50.

(9) Watahiki, M.; Isobe, J.; Kimata, K.; Shima, T.; Kanatani, J. I.; Shimizu, M.; Nagata, A.; Kawakami, K.; Yamada, M.; Izumiya, H.; Iyoda, S.; et al. Characterization of enterohemorrhagic Escherichia coli O111 and O157 strains isolated from outbreak patients in Japan. *J. Clin. Microbiol.* **2014**, *52* (8), 2757−2763.

(10) Byrne, L.; Jenkins, C.; Launders, N.; Elson, R.; Adak, G. K. The epidemiology, microbiology and clinical impact of Shiga toxin-

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX

producing Escherichia coli in England, 2009−2012. *Epidemiol. Infect.* **2015**, *143* (16), 3475−3487.
(11) Collivignarelli, M.; Abbà, A.; Benigna, I.; Sorlini, S.; Torretta, V. Overview of the main disinfection processes for wastewater and drinking water treatment plants. *Sustainability* **2018**, *10* (1), 86.
(12) Jiang, J.; Han, J.; Zhang, X. Nonhalogenated aromatic DBPs in drinking water chlorination: a gap between NOM and halogenated aromatic DBPs. *Environ. Sci. Technol.* **2020**, *54* (3), 1646−1656.
(13) Genisoglu, M.; Ergi-Kaytmaz, C.; Sofuoglu, S. C. Multi-route−Multi-pathway exposure to trihalomethanes and associated cumulative health risks with response and dose addition. *J. Environ. Manage.* **2019**, *233*, 823−831.
(14) Agbaba, J.; Molnar, J.; Tubić, A.; Watson, M.; Maletić, S.; Dalmacija, B. Effects of water matrix and ozonation on natural organic matter fractionation and corresponding disinfection by-products formation. *Water Sci. Technol.: Water Supply* **2015**, *15* (1), 75−83.
(15) Mousel, D.; Palmowski, L.; Pinnekamp, J. Energy demand for elimination of organic micropollutants in municipal wastewater treatment plants. *Sci. Total Environ.* **2017**, *575*, 1139−1149.
(16) Wastewater technology fact sheet: ozone disinfection. U.S. Environmental Protection Agency: Washington, DC, 1999.
(17) Adeleye, A. S.; Conway, J. R.; Garner, K.; Huang, Y.; Su, Y.; Keller, A. A. Engineered nanomaterials for water treatment and remediation: costs, benefits, and applicability. *Chem. Eng. J.* **2016**, *286*, 640−662.
(18) *Municipal Wastewater Disinfection Design Manual*; U.S. Environmental Protection Agency: Cincinnati, OH, 1986.
(19) Metcalf & Eddy; Tchobanoglous, G.; Burton, F. L.; Stensel, H. D.; Eramo, B.; Sirini, P.; Chiavola, A.; Polettini, A.; Pomi, R. *Ingegneria delle acque reflue: trattamento e riuso*; McGraw-Hill, 2006.
(20) Wang, J.; Wu, Y.; Yang, Z.; Guo, H.; Cao, B.; Tang, C. Y. A novel gravity-driven nanofibrous membrane for point-of-use water disinfection: polydopamine-induced in situ silver incorporation. *Sci. Rep.* **2017**, *7* (1), 2334.
(21) Tan, X.; Chen, C.; Hu, Y.; Wen, J.; Qin, Y.; Cheng, J.; Chen, Y. Novel AgNWs-PAN/TPU membrane for point-of-use drinking water electrochemical disinfection. *Sci. Total Environ.* **2018**, *637*, 408−417.
(22) Zeng, X.; McCarthy, D. T.; Deletic, A.; Zhang, X. Silver/Reduced Graphene Oxide Hydrogel as Novel Bactericidal Filter for Point-of-Use Water Disinfection. *Adv. Funct. Mater.* **2015**, *25* (27), 4344−4351.
(23) Hajipour, M. J.; Fromm, K. M.; Akbar Ashkarran, A.; Jimenez de Aberasturi, D.; de Larramendi, I. R.; Rojo, T.; Serpooshan, V.; Parak, W. J.; Mahmoudi, M. Antibacterial properties of nanoparticles. *Trends Biotechnol.* **2012**, *30* (10), 499−511.
(24) Liu, Y.; Tourbin, M.; Lachaize, S.; Guiraud, P. Silica nanoparticles separation from water: aggregation by cetyltrimethylammonium bromide (CTAB). *Chemosphere* **2013**, *92* (6), 681−687.
(25) Huang, Y.; Keller, A. A. EDTA functionalized magnetic nanoparticle sorbents for cadmium and lead contaminated water treatment. *Water Res.* **2015**, *80*, 159−168.
(26) Pina, A. S.; Batalha, Í. L.; Fernandes, C. S.; Aoki, M. A.; Roque, A. C. Exploring the potential of magnetic antimicrobial agents for water disinfection. *Water Res.* **2014**, *66*, 160−168.
(27) Sutton, S. Measurement of microbial cells by optical density. *Journal of Validation Technology* **2011**, *17* (1), 46−49.
(28) Lemire, J. A.; Harrison, J. J.; Turner, R. J. Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat. Rev. Microbiol.* **2013**, *11* (6), 371−384.
(29) *Recreational water quality criteria*; U.S. Environmental Protection Agency Health and Ecological Criteria Division, 2000.
(30) Cliver, D. O.; Foell, W. K.; Goepfert, J. M. Biocidal effects of silver. *Contract NAS* **1971**, 9−9300.
(31) Lin, Y. E.; Vidic, R. D.; Stout, J. E.; Yu, V. L. Negative effect of high pH on biocidal efficacy of copper and silver ions in controlling Legionella pneumophila. *Appl. Environ. Microbiol.* **2002**, *68* (6), 2711−2715.
(32) Butkus, M. A.; Edling, L.; Labare, M. P. The efficacy of silver as a bactericidal agent: advantages, limitations and considerations for future use. *Aqua* **2003**, *52* (6), 407−416.
(33) Johnson, F. H.; Lewin, I. The growth rate of *E. coli* in relation to temperature, quinine and coenzyme. *J. Cell. Comp. Physiol.* **1946**, *28* (1), 47−75.
(34) Kadam, S. R.; den Besten, H. M.; van der Veen, S.; Zwietering, M. H.; Moezelaar, R.; Abee, T. Diversity assessment of Listeria monocytogenes biofilm formation: impact of growth condition, serotype and strain origin. *Int. J. Food Microbiol.* **2013**, *165* (3), 259−264.
(35) Harris, D. C. *Quantitative chemical analysis*; Macmillan, 2010.
(36) Secondary drinking water standards: Guidance for nuisance chemicals; U.S. Environmental Protection Agency Drinking Water Contaminants-Standards and Regulations (last updated January 27, 2020). https://www.epa.gov/sdwa/secondary-drinking-water-standards-guidance-nuisance-chemicals.
(37) Campbell, P. G.; Tessier, A. *Metal speciation in natural waters: Influence of environmental acidification*; American Chemical Society: Washington, DC, 1987.
(38) Zhu, Y.; Liu, H.; Yang, L.; Liu, J. Study on the synthesis of Ag/AgCl nanoparticles and their photocatalytic properties. *Mater. Res. Bull.* **2012**, *47* (11), 3452−3458.
(39) Averill, B.; Eldredge, P. *Chemistry: principles, patterns, and applications*; Recording for the Blind & Dyslexic, 2007.

https://dx.doi.org/10.1021/acsestwater.0c00066
ACS EST Water XXXX, XXX, XXX−XXX