Int. J. Aquat. Biol. (2020) 8(6): 447-454
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Removal of coliform bacteria from dairy wastewater using graphene-silver nanocomposite

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Abstract: Nanoparticles are widely used in removing bacteria from water and sewage. This study evaluated the effect of graphene/silver nanocomposite on the removal of coliform from a dairy effluent. The composition was synthesized and its properties were determined using different techniques such as SEM, XRD and FTIR. The effects of various factors, including pH, adsorbent dose and contact time on coliform removal from solution were studied. Antimicrobial activity of the nanocomposite was examined by pour plate method in a VRBL medium. After preparing the VRBL medium from the sewage effluent, 1 mL of nanocomposite solution and 1 mL of bacterial suspension containing 9×10^4 CFU/mL were added into each of the plates. According to the results, the rate of silver ion release is faster as pH decreases, which naturally results in the increase of disinfection. Moreover, a higher percentage of removal occurred with an increase in contact time and nanocomposite dose due to higher exposure to nanoparticles and their higher penetration into bacterial cell membrane. Therefore, nanoparticles produced by this method exhibited good antibacterial activity, so that 100% of bacteria were eliminated at a nanocomposite concentration of 3.4 g/L and pH=5, after 90 min.

Article history:
Received 9 August 2020
Accepted 27 November 2020
Available online 25 December 2020

Keywords:
Nanocomposite
Coliform bacterium
Removal
Aqueous solution

Introduction

About 80% of diseases in developing countries are caused by poor water quality (Rajaei et al., 2012). Eleven percent of the world's populations still do not have access to clean water resources. Hygiene hazards caused by water pollution in developing countries are much more serious than those in developed countries (Khazaee et al., 2016; Wang and Yang, 2016;Alavian et al., 2017). Some researchers predicted that nearly 135 million people will die by drinking contaminated water by 2020 (Singh et al., 2016). Discharging untreated sewage into environment causes adverse effects to human (Thamilselvi and Radha, 2017) and direct (Mansouri et al., 2013; Mirzajani et al., 2016b; Hamidian et al., 2016) and indirect (Mirzajani et al., 2016a, b) effects on ecosystems. Conventional disinfection methods, including chlorination, ultraviolet, and ozonation are used to prevent or decrease risk of waterborne diseases. The use of chemical disinfectants such as chlorine produces harmful byproducts, when chlorine reacts with other pollutants in water (Mthombeni et al., 2012). Formation of disinfection by-products, such as trihalomethanes is suspected to have a negative effect on human health, especially in terms of carcinogenicity and fetal abnormalities (Hong et al., 2007; Kanan and Karanfil, 2011). Studies over last few decades have indicated a relationship between disinfection of water and formation of disinfection by-products. Thus, it is necessary to identify and use new technologies for water disinfection (Li et al., 2008; Mojoudi et al., 2018, 2019).

Pollutants found in water include organic pollutants, heavy metals, nitrates and radionuclides. In addition, water may contain many problematic organisms, such as bacteria, viruses and parasites, which should be removed or deactivated for the safety of those who are in contact with water (Zarpelon et al.,

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DOI: https://doi.org/10.22034/ijab.v8i6.1025

2016; McCullagh et al., 2007). Despite developments in water management practices, the presence of bacteria and parasite in water is a major threat to human and animal health. *Escherichia coli* is the most important factor in detecting fecal contamination in water resources. Coliform bacteria are considered as indicators of drinking water pollution and known as appropriate indicators of water quality. Therefore, the index of coliform bacteria is now monitored to control microbiological quality in water treatment plants (Wiley and Sonse, 2005).

Production of nanoparticles with antimicrobial properties provides a suitable ground for water decontamination. Nanoparticles with different mechanisms of chlorine remove bacteria, which is of high importance in water treatment (Chitra and Annadurai, 2013). Antimicrobial properties of metal nanoparticles are related to their small size and high ratio of surface to volume, which allows them to react closely with microbial membrane (Ruparelia et al., 2008). Due to their proper surface area and excellent adsorption capacity, silver nanoparticles are widely used for disinfecting water. Several researchers approved antibacterial effects of silver nanoparticles and their potential against a wide range of microbes, including antibiotic-resistant bacteria (Nanda and Saravanan, 2009; Tang et al., 2017). Conventional techniques for producing nanoparticles include physical, chemical and biological methods (Mojoudi et al., 2018). Advantages of biological methods such as lower cost, faster speed, high production and safe environment led to more attention to these methods compared to two other types (Parikh et al., 2008). Based on above-mentioned background, this study investigated the effect of graphene-silver nanocomposite on coliform bacteria in dairy effluents. Moreover, it studied the effects of parameters such as pH, adsorbent dose and duration on the rate of bacterial removal.

Materials and Methods

This study used 5-10 nm dark gray graphene oxide nanoparticles with 99% purity and silver nitrate (Merck, Germany). Universal 320 bench-top centrifuge (Hettich, Germany) were used in the experiment and a UP50H ultrasonic homogenizer (Hielscher, Germany) to homogenize the solid phase of nanocomposite in the solvent. Nitric acid and 0.1 M sodium hydroxside were also used to adjust acidity.

Synthesis of Ag-rGO nanocomposite: To prepare graphene-silver nanocomposite, 1.7 g of silver nitrate was initially dissolved in 100 mL of deionized water and then 0.62 g of NaOH was added into the solution. The resulting brown Ag_2O deposition was filtered and dissolved in 100 mL of ammonia. $[Ag\ (NH_3)_2^+]$ complex was formed in this stage; then, 7.2 g of oleic acid added into the stirring solution. After stirring for 90 min, 0.1 g of graphene oxide was added (Lan et al., 2014). In addition, 90 mL of mango extract was poured into the solution, and it was stirred for half an hour. Finally, it was placed under UV lamp for 8 hours. Then, the solution was centrifuged at 6000 rpm for 15 min and dried at 50°C in an oven for 12 hours. Several techniques such as SEM, XRD and FT-IR were used to verify the synthesis of Ag-rGO nanocomposite

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Disinfection test: Samples of sewage effluents were collected from Pegah Tehran Dairy Co. and used to evaluate the efficiency of the synthesized disinfectant. The samples were collected before entering the chlorination unit (ISO 19458:2006 - Water quality -

Sampling for microbiological analysis, 2006). After transferring to the laboratory, pour plate procedure (mixed culture) was used for microbial cultivation using Violet Red Bile Lactose (VRBL).

The effects of different parameters such as pH, adsorbent dose and contact time on the removal efficiency of the nanocomposite were investigated (pH = 5, 6, 8 and 9 with a volume of 50 mL solution, concentrations of 0.8, 1.7 and 3.4 g/L of composite; contact time = 0, 60 and 90 min). Nitric acid and 0.1 M sodium hydroxide were used to adjust the pH of aqueous solution. After preparing VRBL medium from wastewater, 1 mL of composite solution and 1 mL of bacterial suspension containing 9×10^4 CFU/mL were added into each of the plates. Then it was incubated at 37°C for 24 hours. After cultivating bacteria, Brilliant-green Bile Lactose (BGBL) medium was used to confirm coliform of the grown colonies. Formation of turbidity and gas in Durham tube confirmed the existence of coliform of grown colonies, and finally, colonies were counted (ISO 4832:2006 - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique, 2006). The experiment was conducted with three replicates.

Results

Determination of Ag-rGO nanocomposite properties using SEM images: Scanning electron microscope (SEM) images of Ag-rGO nanocomposite in different sizes are shown in Figure 1. The presence of the reduced graphene oxide-silver nanocomposite is clearly visible. In addition to graphene plates, silver nanoparticles were accumulated in aggregates as seen as a mass due to their small size (Fig. 1).

Analysis of Ag-rGO nanocomposite using FT-IR spectroscopy: Figure 2 shows the peaks of Ag-rGO nanocomposite using FTIR spectroscopy. Peaks of OH and C=O symmetric stretching vibration and C-O-C asymmetric stretching vibration, as well as C=C binary bond appeared at 3637, 1759, 1051, and 1655 cm^{-1}, respectively. Since this study aimed to reduce graphene oxide to graphene using mango extract, width of OH spectrum was reduced by decreasing graphene.

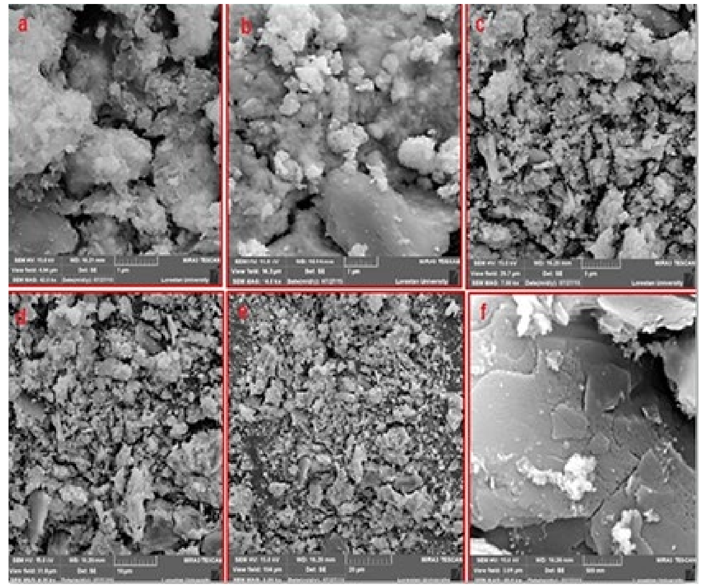

Figure 1. Different magnifications of SEM image of Ag-rGO nanocomposite.

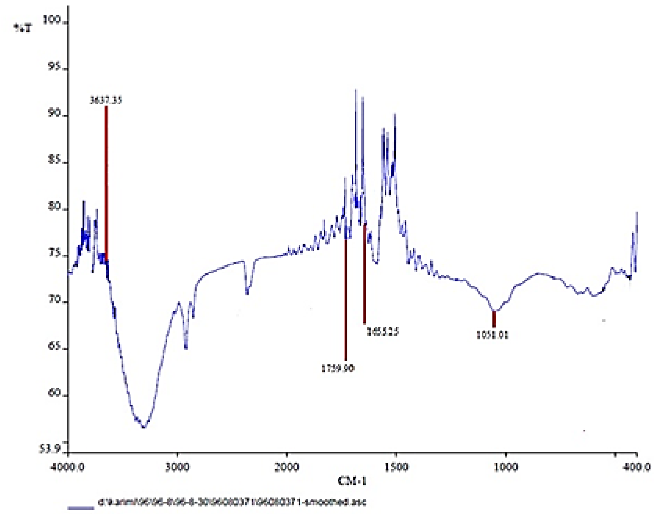

Figure 2. FT-IR spectrum of Ag-rGO nanocomposite: the peaks of OH, C=O, C-O-C and C=C bonds appeared at 3637, 1759, 1051, and 1655 cm^{-1}, respectively.

Analysis of Ag-rGO nanocomposites using XRD pattern: XRD patterns of graphene/silver nano-composite is portrayed in Figure 3. The results of infrared spectroscopy confirmed the presence of C-C bonds on the surface of graphene/silver nanoparticles. Characteristic peaks of the silver nanoparticles were observed in 400-800 nm. Moreover, true peak in a nanometer wave number could be a reason for the existence of the nanoparticles in nanocomposite.

Effect of pH on the efficiency of silver/graphene

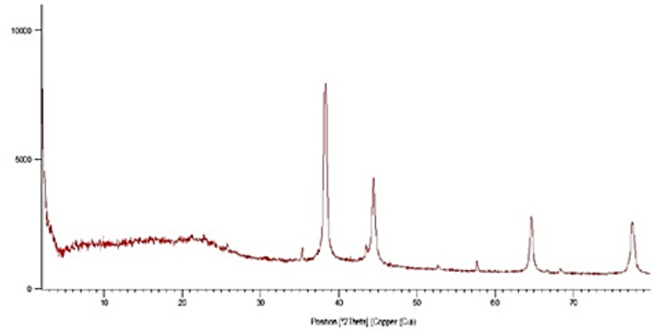

Figure 3. XRD pattern of Ag-rGO nanocomposite: the peaks shown in 400-800 nm confirm the proper formation of nanoparticles.

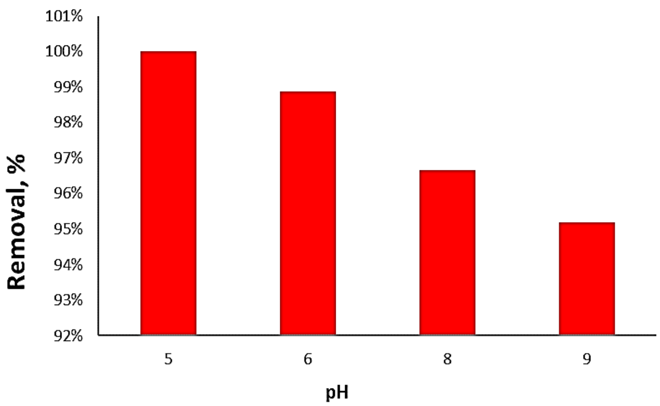

Figure 4. The effect of pH on the removal of coliform bacteria from aqueous solution by Ag-rGO nanocomposite at pH = 5, 6, 8, and 9. The minimum and maximum removals were observed at pH 5 and 9, respectively.

nanocomposite in coliform removal: The efficiency of Ag-rGO nanocomposite for the removal of coliform bacteria increased by a decrease in pH (Fig. 4). Maximum adsorption capacity was observed at pH=5. Figure 5 depicts adsorption capacity of graphene-silver nanocomposite for coliform bacteria. Adsorption capacity of Ag-rGO nanocomposite increased by decreasing pH, and maximum adsorption capacity was observed at pH=5.

Effect of time on the removal of coliform bacteria by graphene-silver nanocomposite: The effect of time on the percentage of coliform removal from solution using graphene-silver nanocomposite is shown in Figure 6. As time increased, adsorption capacity increased and the highest removal percentage was observed after 90 min. Figure 7 indicates adsorption capacity of graphene-silver nanocomposite in removing coliform bacteria from solution. Maximum adsorption capacity was found at 90 min.

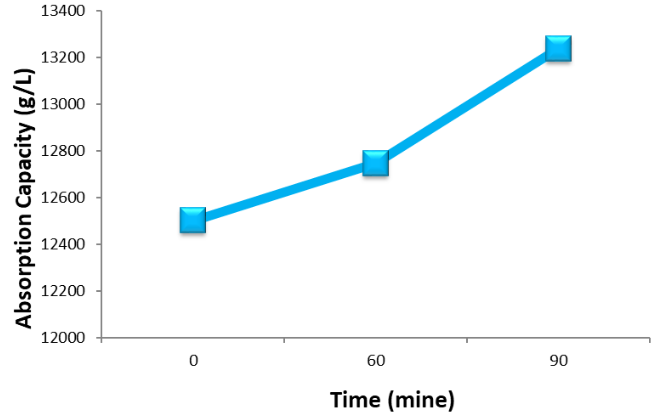

Figure 5. Adsorption capacity of Ag-rGO nanocomposite against the coliform bacteria at pH = 5, 6, 8, and 9. The minimum and maximum removals were observed at pH 5 and 9, respectively.

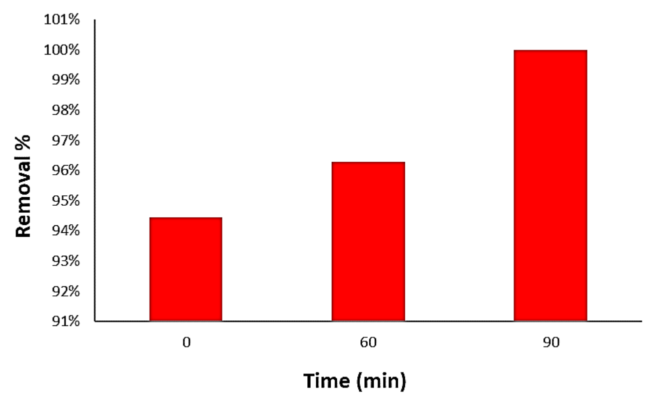

Figure 6. The effect of exposure time on the removal of coliform bacteria from aqueous solution by Ag-rGO nanocomposite at 0, 60 and 90 minutes. The minimum and maximum removals were observed at 0 and 90 minutes, respectively.

Effect of adsorbent dose on the removal of coliform bacteria using graphene-silver nanocomposite: This study was conducted using concentrations of 0.8, 1.7 and 3.4 g/L of nanocomposites and constant concentrations of coliform colonies (9×10^4CFU/mL) in a fixed volume of solution (50 mL) and after 0, 60 and 90 minutes. Percentage of the removal of coliform bacteria was increased by increasing the dose of nanocomposite in solution. The highest removal percentage was observed in the adsorbent dose of 3.4 g/L (Fig. 8). Figure 9 depicts absorption capacity of graphene-silver nanocomposite in different doses for removing coliform bacteria. As nanocomposite dose increased, the absorption capacity increased.

Discussions

Based on the results, bacterial removal efficiency

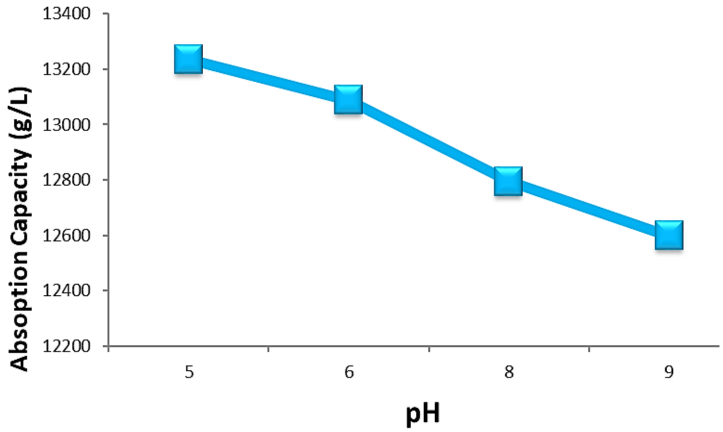

Figure 7. Adsorption capacity of Ag-rGO against coliform bacteria during time contact of 0, 60 and 90 minutes. The minimum and maximum removals were observed at 0 and 90 minutes, respectively.

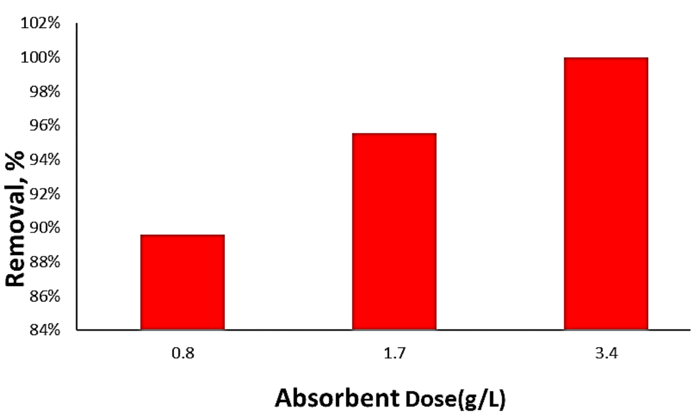

Figure 8. The effect of nanocomposite dose on the removal of coliform bacteria from aqueous solution by Ag-rGOnanocomposite at concentrations of 0.8, 1.7 and 3.4 g/L of nanocomposite and 96 × 104 CFU/mL of bacterial suspension. Minimum and maximum removals were observed at concentrations of 0.8 and 3.4 g/L, respectively.

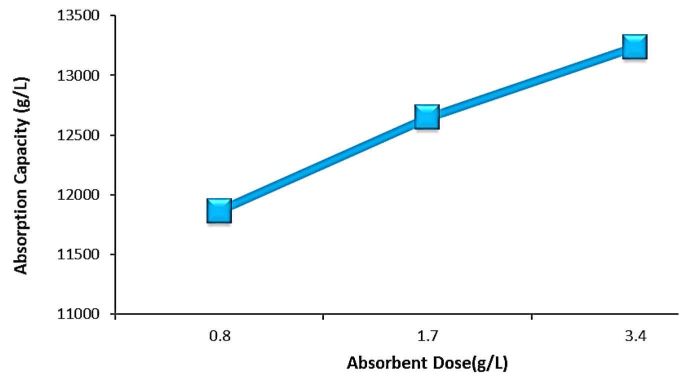

Figure 9. Adsorption capacity of Ag-rGOnanocomposite against coliform bacteria at concentrations of 0.8, 1.7 and 3.4 g/L of nanocomposite and 96 × 104 CFU/mL of bacterial suspension. Minimum and maximum removals were observed at concentrations of 0.8 and 3.4 g/L, respectively.

increased by decreasing pH, so that the maximum removal rate of coliform bacteria was observed at pH=5. Song et al. (2016) conducted a study on the removal of *E. coli* and *Staphylococcus aureus* using graphene-silver nanocomposite at pH=5.5 to 5.8. They found that the highest percentage of bacterial removal is observed at pH=5.5. Shao et al. (2015) also studied antibacterial activity of silver/cellulose nano-composites for removing *E. coli*, *S. aureus*, *Bacillus subtilis*, and *Candida albicans* at pH=5.5 to 5.8. Their study demonstrated that the highest removal efficiency is occurred at pH=5.5. Li et al. (2018) found that graphene-based nanocomposites at pH=5 have the highest disinfection efficiency of *E. coli* and *S. aureus*. In general, pH has a significant effect on the use of nanocomposites during the removal process of coliform bacteria. Silver nanoparticles (AgNPs) are converted into silver ions in aqueous solutions. The amount of free silver ion has a direct relationship with the amount of AgNP oxidation. It is notable that oxidation of these particles is higher in aqueous solutions with lower pH. Silver ion causes the death of these microorganisms by contacting bacteria and damaging cell membrane. Therefore, lower pH leads to the greater and faster release of silver ions and naturally results in the increase of disinfection property (Shao et al., 2015; Li et al., 2018).

Another important factor in the removal process of coliform bacteria is the time to expose organic waste with disinfectant. In our results, the bacterial removal rate increased as time increased, so that the highest percentage of bacterial removal was observed at 90 min. Chang et al. (2016) compared antimicrobial behavior of silver-coated carbon nanocomposites against *E. coli* and *S. aureus* at a time duration of 0 to 25 min indicating that bacterial removal increased as the time increased and maximum (99.99%) bacterial removal was obtained after 25 min. Biswas and Bandyopadhyaya (2016) investigated disinfection of water using activated carbon impregnated with silver nanoparticles. They sought to remove *E. coli* bacteria with a constant number of bacterial colonies (10^4CFU/mL) over a period of 0 to 30 min. Their results showed that maximum amount of bacteria removal is occurred within 30 min with a adsorption does rate of 29.8 μg/L. Moteriya et al. (2017) examined the composition of silver nanoparticles synthesized with leaf of *Cassia roxburghii* against fungi, gram-positive, and gram-negative bacteria. They conducted their experiment at contact time of 0 to 120 min. Their results showed that the removal process of bacteria experienced an increasing trend as time increased, however it did not significantly change from 30 to 120 min. Zazouli et al. (2016) studied deactivating *E. coli* bacteria in water via silver nanoparticles with ultraviolet radiation over a period of 10 to 60 min. It was found that bacterial removal process increased as the contact time increased, so that maximum efficiency (100% removal of *E. coli* bacteria) was achieved at 60 min. In general, when the contact time increases, the amount of nanoparticles binding to the bacteria and their penetration into the cell increase. Moreover, by increasing the contact time more silver ions are produced and thus the bacterial removal process increases.

The results of antimicrobial property determination of Ag-rGO nanocomposite showed that there is a direct relationship between concentration of nanocomposite and percentage of bacterial removal. The higher concentration of nanocomposite in solution results in the higher removal rate of coliform. In the present work, the highest removal efficiency was observed at a concentration of 4.3 g/L. Zhang et al. (2016) used 25.6 to 50 μg/mL of separated silver nanoparticle-decorated magnetic graphene oxide to remove *E. coli* and *S. aureus* from a solution. Their results suggested that the removal percentages of these bacteria increase by increasing adsorbent dose. The highest removal percentage applying an adsorbent dose of 50 μg/mL was 99.99 and 99.96% for *E. coli* and S. *aureus*, respectively. Parandhaman et al. (2015) investigated antimicrobial behavior of silica-silver nanocomposite in disinfecting gram-negative bacteria of *E. coli* and *Pseudomonas aeruginosa*. Antibacterial activity of this nanocomposite was examined at a dose range of 0.1 to 2 μg/mL. Thepr results indicated that increasing the adsorbent dose increases the bacterial removal efficiency, so that the highest removal percentage of bacteria with an adsorbent dose of 2 μg/mL was 99.98 and 99.73% for *E. coli* and *S. aureus*, respectively. Chandraker et al. (2017) studied antibacterial properties of amino acid functionalized silver nanoparticles decorated on graphene oxide sheets. Antibacterial activity of this nanocomposite was performed for gram-negative bacteria of *E. coli* and gram-positive bacteria of *S. aureus* with an adsorbent dose of 20 to 100 μg/mL. Based on their results increasing the selected adsorbent dose increases antibacterial activity, so that the highest bacterial removal (100%) was achieved in the adsorbent dose of 100 μg/mL for both *E. coli* and *S. aureus*.

Conclusion

This study investigated the effect of graphene-silver nanocomposite on the removal of coliform bacteria from dairy effluents. Analysis of different variables on the antibacterial behavior of Ag-rGO nanocomposite indicated that there is an increasing trend in the removal of coliform bacteria by increasing pH, so that the highest removal efficiency of coliform bacteria was observed at pH=5. Moreover, as time increased, an increasing trend was observed in the removal of coliform bacteria, so that the highest bacterial removal process occurred at 90 min. As the selected dose increased, an increasing trend occurred in the removal of bacteria, and the highest removal rate of coliform bacteria was observed in the adsorbent dose of 3.4 g/L. In general, graphene-silver nanocomposite has high ability to remove coliform bacteria, and its disinfectant activity has a direct relationship with pH of solution, exposure time and disinfectant dose.

Abbreviations:

Trihalometanes (THM); Ultrasonic Homogenizer (UH), Crystal violet neutral red bile lactose agar (VRBL), Brilliant green lactose bile broth (BGBL), Scanning Electron Microscope(SEM), Fourier Transform Infrared Spectroscopy (FTIR), and. X-Ray Diffraction (XRD).

Acknowledgement
This manuscript was supported by Iran National Science Foundation (INSF) under the contract No. 97002416 and Chinese Academy of Science President's International Fellowship Initiative. Grant No. 2021VEA0004.

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