Inactivation of Enterococcus Faecalis in Drinking Water using Silver Nanoparticles Embedded Paper

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Abstract

In this paper, a cellulose paper was impregnated with silver nanoparticles (AgNPs) for the purpose of removing Enterococcus faecalis from drinking water. AgNPs papers were prepared by chemical reduction of silver nitrate (AgNO3) with various concentrations (0.005 M, 0.01 M, 0.015 M, and 0.025 M) using sodium borohydride (NaBH4) as a reducing agent. Two ratios of NaBH4/AgNO3 of 2:1and 10:1 were used to show the effect of reduction on the formation and removal efficiencies of AgNPs. AgNPs papers were characterized using SEM and TEM. TEM images showed that the silver nanoparticles size in the papers varies from 1.3 to 75 nm.

Keywords: *Enterococcus Faecalis , Drinking Water, Silver Nanoparticles.*

Introduction

Disinfection of potable water is the specialized treatment for destruction or removal of organisms capable of causing disease; it should not be confused with sterilization, which is the destruction or removal of all life [1]. Although disinfection methods currently used in drinking water treatment can effectively control microbial pathogens, researches in the past few decades have revealed a dilemma between effective disinfection and formation of harmful disinfection byproducts (DBPs) [2]. Three categories of human enteric pathogens are of concern in drinking water: bacteria, viruses, and amebic cysts. Disinfection must be capable of destroying all three [3]. Destruction or removal of these organisms is essential in providing a safe potable water supply. Some bacteria, viruses, protozoa, and larger organisms ingested from contaminated water cause diseases varying from mild illnesses to life-threatening [1]. The Enterococcus genus is placed in the Enterococcaceae family and consists of species that occur in human and animal gastro-intestinal (GI) tracts, as well as in the guts of insects traditional fermented food and dairy products, and in various environments including plants, soil and water [4]. Enterococcus faecalis is a non-spore-forming, fermentative, facultatively anaerobic, Gram-positive coccus. Enterococcus faecalis cells are ovoid and 0.5 to 1 μm in diameter. They occur singly, in pairs, or in short chains, and are frequently elongated in the direction of the chain. They typically have an optimum growth temperature of 35°C and a growth range from 10 to 45°C [5]. They currently rank among the most prevalent multidrug resistant hospital pathogens worldwide as the third most commonly isolated healthcare pathogen, and are capable of causing a variety of infections including endocarditis, sepsis, surgical wound infections, and urinary tract infections [6]. The organism has the natural ability to acquire, accumulate and share extrachromosomal elements encoding virulence traits, which help to colonize, compete with other bacteria, resist host defense mechanisms and produce pathological changes directly through production of toxins or indirectly through induction of inflammation [5]. Nanotechnology and its application is one of the rapidly developing sciences [7]. Silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms [8].

Experimental Procedure

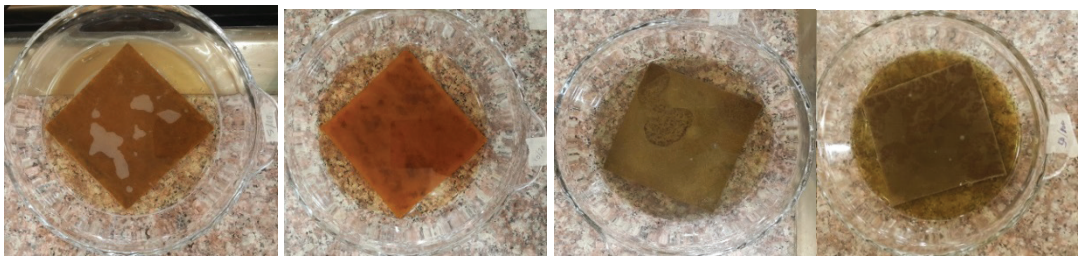
Sampling

The samples were taking from Shatt al-Hilla, at Al-Hilla city/Iraq and during the period (November 2018 – March 2019). 500 ml of water as grabbed and kept in precleared plastic bottle. The samples were analyzed immediately to prevent any change in their quality that

may occur.

Preparation of AgNPs papers

A (10 cm * 10 cm * 0.8 mm) off-white paper, 100% alpha cellulose was used to be embedded with silver nanoparticles. AgNPs papers were prepared by in situ reduction of AgNO3 with various concentrations (0.005 M, 0.01 M, 0.025 M and 0.05 M) with two reduction ration of 2:1 and 10:1. Each paper was soaked in 40 ml of AgNO3 solution for 30 minutes, then it was washed with ethanol for 1 minute to remove the excess Ag ions which not absorbed by the paper. To form AgNPs, the paper was placed in 40 ml of NaBH4 solution for 1 hr. After that, the paper was soaked in de-ionized water for 30 minutes. Then the paper was dried in the oven at 60 ℃ for 2.5 hrs.



(a)

(c)

Fig. 1: (a) AgNPs paper during preparation with NaBH4/AgNO3 ratio of 2:1. (b) AgNPs paper during preparation with NaBH4/ AgNO3 ratio of 10:1.

Characterization

The synthesized AgNPs papers were characterized by Scanning Electron Microscopy (SEM), type Quanta 450 available at the University of Babylon/ College of Pharmacy and Transmission Electron Microscopy (TEM) available at Al-Nahrain University/ College of Medicine.

Acid Digestion

To determine the silver content in the AgNPs paper, an acid digestion of the paper was performed and then analyzes the amount of dissolved silver with an Atomic Absorption Spectrometer (AAS) (AA320N) available at the University of Babylon/ College of Material Engineering. Approximately a 100 mg of the dried AgNPs paper was reacted with 5 ml of nitric acid (HNO3) and 5 ml of water. The mixture was boiled until the paper was disintegrated. 5 ml of 30% hydrogen peroxide (H2O2) was added to the mixture to assist in the complete oxidation of the organic matter to release additional metals into the solution. The mixture was boiled again and left to be cooled, then filtered through a filter paper and then diluted by adding a 100 ml of water. The diluted mixture was tested for silver content using an AAS.

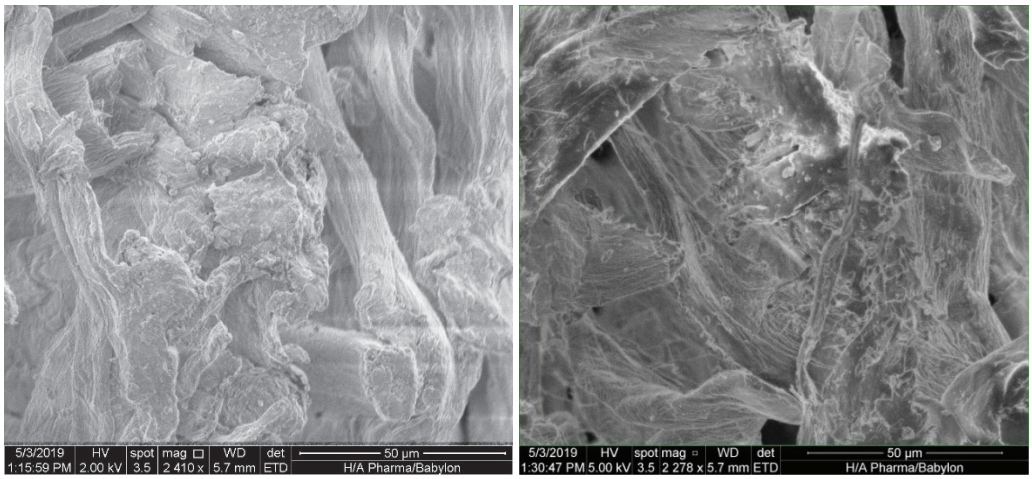
Microbiological Test

The raw water samples were cultured using serial dilutions method, 1 ml of the sample was diluted in 9 ml of distilled water (1:10 dilution). 1 ml of 1:10 dilution mixed with 9 m; of distilled water (1:100 dilution), etc. the filtered water samples were cultured without dilution. 0.1 ml of each sample was spread over a media plate and then the plates were incubated in 37 ℃ for 48 hrs in an incubator (LIB-030M).

Results and Discussion

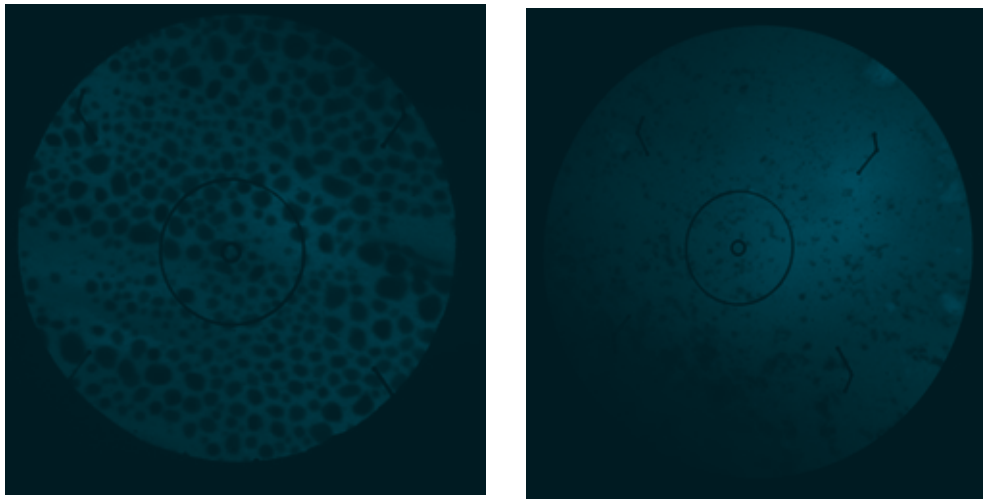
Paper Characterization

The AgNPs papers were characterized by SEM and TEM. Fig.2 represents the images obtained by SEM to show the presence of AgNPs in paper fibers. Fig. 3 represents the images obtained by TEM to determine the particles sizes of AgNPs. Table 1 represents the particles sizes of AgNPs obtained by TEM test.



(a) (b)

Fig. 2: Images obtained from SEM. (a) 2:1 $NaBH_4/AgNO_3$ ratio. (b) 10:1 $NaBH_4/AgNO_3$ ratio.



(a) (b)

Fig. 3: TEM images. (a) 2:1 $NaBH_4/AgNO_3$ ratio. (b) 10:1 $NaBH_4/AgNO_3$ ratio.

Table 1: The particles sizes of AgNPs obtained by TEM test

AgNO3 concentration, M	Nanoparticle Size Range ,nm	
	2:1 NaBH4/AgNO3 ratio	10:1 NaBH4/AgNO3 ratio
0.005	6.86 - 75	2.028 – 39.395
0.01	3 – 69.26	3 – 26.833
0.025	1.414 – 32.802	1.333 – 27.659
0.05	2 – 21.84	0.943 – 20.044

TEM images showed that an excess of sodium borohydride reductant (10:1 ratio of sodium borohydride to silver nitrate) gave more uniform and smaller nanoparticles.

Acid Digestion

Acid digestion was performed to determine the silver content of the paper. The results were obtained by using AAS (AA320N). Table 2 shows the results of the AAS test.

Table 2: Silver content of each paper

AgNO3 concentration, M	Silver content (mg Ag/g of dried paper)	
	2:1 NaBH4/AgNO3 ratio	10:1 NaBH4/AgNO3 ratio
0.005	3.9584	4.3426
0.01	4.4765	4.5505
0.025	6.3268	6.9206
0.05	7.8669	8.7691

The acid digestion of AgNPs papers showed silver content ranging from 3.9 to 8.7 mg Ag per dry gram of paper. The increase in silver content of the paper correlates with the increase in precursor silver ion concentration of the solution in which the papers were soaked, prior to reduction. For the same concentration of AgNO3, the NaBH4/AgNO3 ratio of 10:1 resulted in more silver content than 2:1 ratio.

Bactericidal Effectiveness of AgNPs papers

Fig. 4 shows the effect of the silver content in the AgNPs paper on the removal efficiency of Enterococcus faecalis of filtered water samples and raw water samples with a NaBH4/AgNO3 ratio of 2:1 and 10:1 respectively.

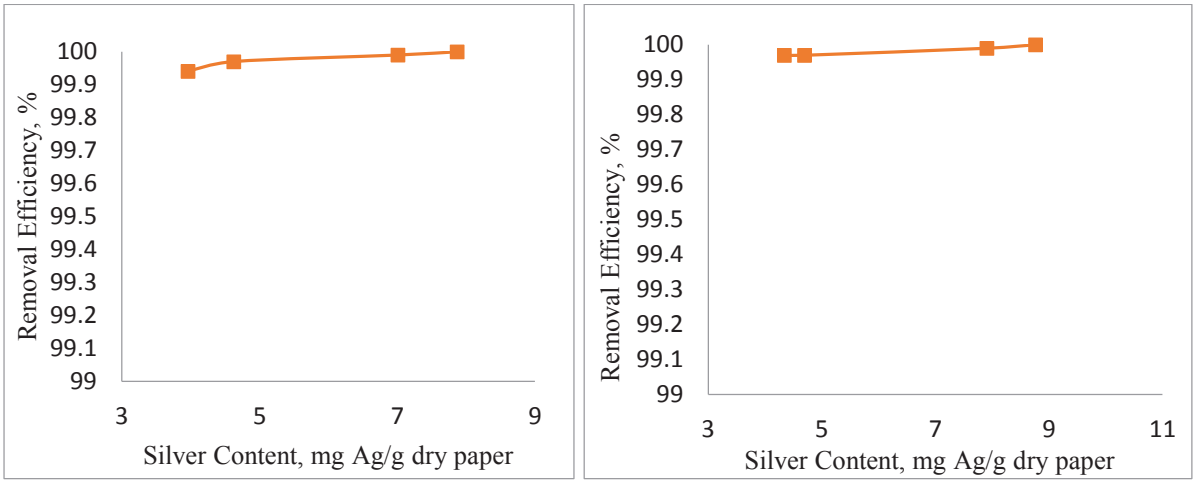


Fig. 4: Effect of silver content on the removal efficiency of Enterococcus faecalis of raw water samples with a: (a) NaBH4/AgNO3 ratio of 2:1. (b) NaBH4/AgNO3 ratio of 10:1.

Fig. 4 shows that removal efficiency Enterococcus Faecalis of the raw water samples for both ratios ranges from 99.9 % to 100% for all silver contents.

Analysis of silver content in The Effluent

Due to possible human health effects from silver exposure, the silver content in the effluent water was analyzed by AAS. Table 4 represents relationship between the silver content in the paper and silver release in the effluent.

Table 4: The relationship between the silver content in the papers and silver in the effluent water.

AgNO3 concentration, M	NaBH4/AgNO3 ratio	Silver Content in the Effluent, mg/L
0.005	2:1	0
	10:1	0
0.01	2:1	0
	10:1	0
0.025	2:1	0.021
	10:1	0.043
0.05	2:1	0.043
	10:1	0.082

As shown in Table 4, the average silver content in the effluent water for the three replicates range from 0 to 0.082 which meets the United States- Environmental Protection Agency (US-EPA) guideline for drinking water of less than 0.1 mg/L [EPA, 2018]. This was due to the stability of silver nanoparticle in the cellulose paper. Sodium borohydride acts not only a reducing agent but also as an ion stabilizer, which prevents silver ions from aggregation. Moreover, hydroxyl and ether groups in the cellulose fiber play an important role in the stabilization of metal nanoparticles.

Conclusions

Silver nanoparticles used in this study were well dispersed and stabilized on the paper fibers. Chemical reduction of AgNO3 by using NaBH4 as a reducing agent resulted in spherical silver nanoparticles. The NaBH4/AgNo3 ratio of 10:1 resulted in smaller sizes of silver nanoparticle and more silver content than the ratio of 2:1 for the same AgNO3 concentration. (99.9-100)% inhibition of Enterococcus Faecalis was obtained with all the concentrations of AgNO3 and NaBH4 and for both NaBH4/AgNo3 ratios.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Water Resources Engineering/ Al-Qassim Green University and all experiments were carried out in accordance with approved guidelines.

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