Nanocomposite filter made from porous mineral tuff with absorbed silver nanoparticles and its application for disinfection of water

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ABSTRACT

Nanocomposite filters were developed by sorption of silver (Ag) nanoparticles into a matrix of household filters consisting of porous mineral tuff and their subsequent chemical deposition. The antimicrobial effects of the nanocomposite filter with Ag nanoparticles on *Escherichia coli* and *Enterococcus faecalis* and on a mixture of soil microorganisms were shown in water. The level of influence of Ag directly depended on its quantity to cause an inhibitory effect on bacteria; for each type of bacteria the amount of Ag causing antibacterial effect varied. Moreover, the efficiency of the filter for large-volume disinfection of water (up to 1,000 L) was determined. It depended on Ag nanoparticles' quantity and the amount of microbial load. Nanocomposite filters from porous mineral tuff with adsorbed Ag nanoparticles have antibacterial properties and can be recommended for disinfection of a large volume of drinking water.

Key words | Ag nanoparticles, antibacterial effect, large-volume disinfection of water, nanocomposite filter, water supply

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INTRODUCTION

For thousands of years water quality has been steadily deteriorating. Now, water has reached such levels of pollution that its use for different purposes has become very limited and jeopardizes human health. According to epidemiological data of the CDC (Centers for Disease Control and Prevention. Data available from www.cdc.gov/mmwr/preview/mmwrhtml/ss5512a4.htm) (Liang *et al.* 2006), in the United States, most cases of outbreaks of gastrointestinal diseases are associated with drinking water. By the WHO's estimation, the disease burden from water, sanitation and hygiene is 4.0% of all deaths and 5.7% of the total disease burden occurring worldwide (Prus *et al.* 2002).

The issue of filtration and disinfection of water used by people for different purposes has become urgent. Nowadays, various household filters are available. Depending on the type of purification the filters relate to different categories such as mechanical, ion exchange, reverse osmosis systems, physical-chemical, electrical (Jacobsen 2004; Stubbe *et al.* 2016).

Most of the filters perform purification on a certain range of contaminations, so in order to achieve more efficiency of results a combination of multiple filters performing various types of purification are used or filtration systems are constructed that require significant costs regarding installation and subsequent maintenance. Another considerable disadvantage of filters performing purification of water with these methods is that the filters become a source of water contamination due to microorganisms that can accumulate and multiply in the filters with irregular water supply. Additional disinfection of drinking water or the use of filters having antibacterial properties can provide a solution to that problem.

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Disinfection of water is carried out by chemical and physical methods. Among the physical methods of decontamination the following are known: boiling, ultrasonic treatment, impact by electric discharge, ultraviolet irradiation (Lantagne & Clasen 2012; Sharma & Bhattacharya 2017). Chemical methods of disinfection include water treatment with strong oxidants: ozone, chlorine-containing substances, oligodynamic effect (influence with heavy metal ions – Ag, Cu, and others) (Crump *et al.* 2005; Pradeep 2009). Usually, for drinking water disinfection, a combination of different methods such as photocatalysis (Liu *et al.* 2016; Thakare & Ramteke 2017) have been applied.

Bactericidal properties of silver (Ag) and its compounds have been known since ancient times (Lok *et al.* 2007). It has been shown that Ag has an inhibitory effect on bacteria, and this action of Ag is stronger than that of other metals (Zhou *et al.* 2012; Lemire *et al.* 2013). There are data showing that the sensitivity of various microorganisms to Ag is not the same (Feng *et al.* 2000; Vardanyan *et al.* 2015).

The works of other researchers have demonstrated that the antibacterial effect of Ag is greater the higher the concentration of Ag ions (Kim *et al.* 2007). Also proven is the fact that the presence of an antibacterial effect of Ag is included in the composition of different matrices, thereby expanding the range of its application (Azócar *et al.* 2012; Palza 2015; Song *et al.* 2016).

Research was implemented to improve the filter cartridge, which would carry out several water treatment methods, in particular decontamination. Adding Ag nanoparticles to the filter may allow the development of a nanocomposite filter, which may have antibacterial properties, the study of which was the purpose of the present paper.

MATERIALS AND METHODS

Filters

The research filter cartridges used were made from tuff, which is an ecologically pure, natural, porous mineral (Yervandakert stone depositary, Armenia).

The cartridges have a hygiene certificate (No. 72 from 22.09.1999, Ministry of Health of the Republic of Armenia) and received an expert sanitary-epidemiological report (No. 77.99.10.234.D.004597.06.04 from 19.11.2001) of the Scientific Research Institute of Human Ecology and Environmental Hygiene after A.N. Sysin (now the Center for Strategic Planning and Management of Medical and Biological Health Risks, Ministry of Health of Russian Federation, Moscow). The report was based on the results of testing, which included the following areas of research: the chemical composition of the filter by spectral analysis, migration of chemical substances from the filter (aqueous extracts), content of radioactive components in the filter and aqueous extracts, efficiency of water treatment from chemical compounds of iron, efficiency of water treatment by organoleptic parameters and complex assessment of quality of aqueous extracts from the filter, based on bio-testing results on hydrobionts. According to the conclusion, the chemical composition of the filter (tuff) was determined, and the following impurities were found in it: silicone (as SiO_2 – 10%), aluminum (as Al_2O_3 – 4.18%), magnesium (as MgO – 0.82%), calcium (as CaO – 2.53%), iron (as Fe_2O_3 – 1.23%), manganese (as MnO – 0.032%), titan (as TiO_2 – 0.13%), strontium (as SrO – 0.041%), barium (as BaO – 0.068%). Importantly, the following chemical elements, which may have toxic effects, were not detected (below the level of detection): beryllium, nickel, cobalt, vanadium, chrome, lead, zinc, boron, thorium, uranium, bismuth, antimony, phosphorus, lithium, lanthanum, zirconium. Moreover, the filter did not deteriorate the organoleptic properties of water (absence of smell and taste in water extracts, no change in the amount of chromaticity in comparison with the control).

Thus, the filters were designed for preliminary micro-purification of drinking water from mechanical particles, reducing turbidity (88–65%), color (85%), and iron content (70%). A filter has porosity, i.e., it performs *mechanical* filtration. At the same time, tuff is a mineral with high sorption properties (Cho *et al.* 1997; Lee *et al.* 2006), therefore the filtration is also performed by the *sorption* of harmful substances dissolved in water. Nevertheless, water that passed through this filter requires an additional disinfection.

Preparation of filter

Filters were authorized for use in household and drinking water supplies. At the first stage, large pieces of tuff were crushed to granules of different sizes. Then, after passing through a sieve with different sizes of holes, the granules were differentiated in size.

The first stage of the filter cartridge preparation process is performed by mixing certain sizes of crushed tuff granules with liquid glass (sodium silicate). Depending on the ratio of the fractions of the tuff granules in the mixture, cartridges with different density and hence with different porosity are obtained. Then, the mass received a certain form such as a cylinder. A gravimetric press and a vibrator are used during formation. The central axis of the cartridge is formed by using a central rod. Depending on the ratio of the granule fractions and the value of gravimetric pressure, the density of the cartridge and the pore sizes may vary. As a result of vibration, the granules of tuff are packaged in the cartridge in such a way that the smaller fraction is distributed in the central part of the cartridge, and the larger fraction is distributed to the outer part of the cartridge; consequently, the density of the structure of the cartridge increases toward the center, and the pore sizes, inversely to density, decrease towards the center.

This mixture is formed and then immersed in a calcium chloride solution. When liquid glass and calcium chloride come into contact within the pores of the formed mixture, a chemical process of silication occurs resulting in the granules of tuff to be bonded to each other which gives the cartridges strength (Lagaly *et al.* 2000). During the last stage of the filter cartridge preparation process, in order to get rid of excess calcium chloride, sodium chloride and other substances, as formed during the chemical reaction, the cartridge is processed with hot water. They are then dried and are ready to use.

Cartridges with the highest density of structure were used, which could be manufactured without violating the integrity of the cartridge, taking into account the water pressure in the pipeline, to ensure the filtration of water through the cartridge without additional expenditure of energy.

The cylindrical cartridges used measured 248 mm in height, 62 mm in diameter, and weighed about 1.2 kg (Figure 1). The filtering surface area of the cartridge was calculated to be 482.8064 cm^2. To both ends of the cartridge special caps were attached, with which the cartridge was fixed in the filter flask. Since they did not take part in filtration they were not included in the calculation of the filtering surface of the cartridge.

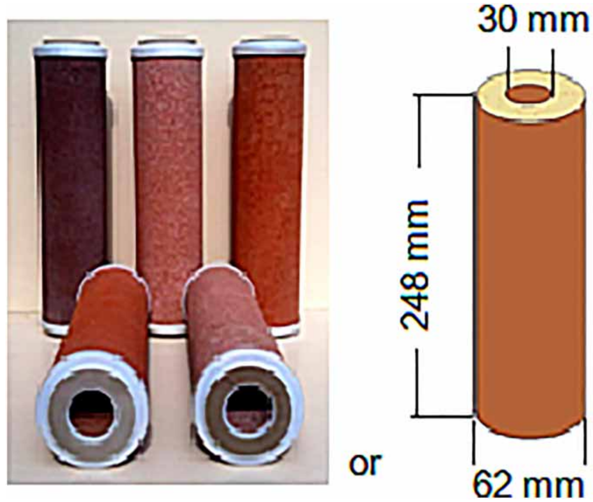

Figure 1 | The filter is made from tuff (right).

Silver nanoparticles

Ag nanoparticles were obtained by electrochemical synthesis (Rodriguez-Sanchez *et al.* 2000). The sizes of Ag particles varied within a nanometer (Figure 2).

Preparation of nanocomposite filters

At the stage of the cartridge mixture preparation, Ag nanoparticles were added to the filter in an amount of 0.1 g or 0.3 g per one cartridge, without any technological changes in the manufacturing process, so as not to cause structural changes in the cartridge (pore sizes, cartridge density).

Interaction of Ag nanoparticles with the filter

To determine the strength of the interaction of Ag nanoparticles with the tuff which was inversely proportional to the removal (leaching) of the Ag from the filter, purified and sterilized by filtration (0.22 μm filter) water was passed

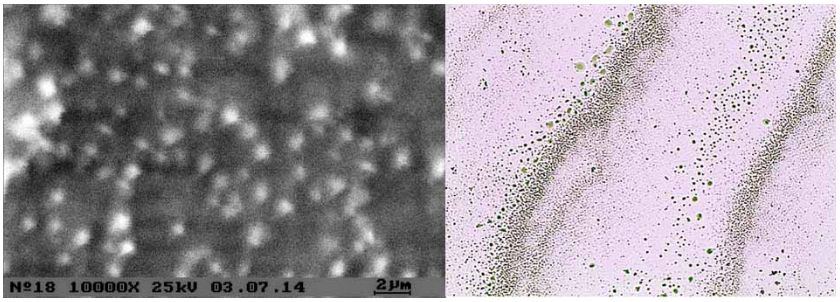

Figure 2 | Electron microscopy of colloidal silver obtained by electrochemical synthesis; 10,000× magnification (left). Microscopy with light microscope; 1,000× magnification (right).

through the filter containing Ag nanoparticles (0.3 g). After filtration of 5 L, 10 L, or 20 L of water, samples were taken, which together with the initial water sample were tested for the content of Ag or its compounds by flame method (acetylene-air) using atomic absorption spectrophotometer (Shimadzu SN: AA-7000/AAC, Japan). The results showed the absence of even trace amounts of Ag in all filtrates. The lower limit of sensitivity of the atomic absorption spectrophotometer was 0.2 mg/L. It suggested that Ag nanoparticles were well fixed in the mineral.

Bacteria studied

Selection of microorganisms was carried out based on the structure of the bacterial cell wall due to the impossibility of studying the effect of nanocomposite filter on the whole spectrum of microorganisms. It was found that the impact of Ag nanoparticles on Gram-positive and Gram-negative bacteria differs from each other (Jung *et al.* 2008). Accordingly, experiments were carried out both on Gram-positive bacteria *Enterococcus faecalis* (*E. faecalis*) American Type Culture Collection (ATCC)29212, and Gram-negative bacteria *Escherichia coli* (*E. coli*) ATCC25922 (laboratory stock strain). Besides the differences in the structure of the cell wall, these bacteria are of interest as indicators of the sanitary quality of drinking water (European Commission 1998).

Antibacterial activity of the filter

To detect the presence of antibacterial properties of the nanocomposite filter, a modified version of disk diffusion method was used (EUCAST 2015). For this purpose, the surface of the nutrient medium was inoculated with a suspension of test bacteria *E. coli* (10^6 colony forming unit (CFU)/mL), which was prepared from overnight growth. Its density was determined with a spectrophotometer at wavelength 625 nm in 10^8 CFU/mL (0.5 McFarland). After inoculating and drying the plate, the pieces of nanocomposite filter containing 0.1 g of Ag, and filter pieces without Ag wetted with sterile purified water were placed on the surface. Then, agar plate was incubated at 37°C for 24 h. A 2–3 mm zone of growth inhibition of test microbes could be clearly seen around pieces of the filter with Ag after incubation (which remained unchanged even after 7 days of incubation), indicating the presence of antibacterial properties of the filter, and pieces of filter without Ag have not caused growth inhibition of the test-microbe.

Antibacterial properties of the filter towards certain microorganisms

Five liters of water contaminated with *E. coli* and 5 L of water contaminated with *E. faecalis* were passed through the filtration system assembled in the laboratory.

Purified water sterilized by filtration through a 0.22 µm filter was used (Milli-Q Direct8, Millipore, France, Sn: F1NA27613B), and according to the ASTM (American Society for Testing and Materials), has parameters corresponding to Type 1, which meets the requirements for microbiological analyses (NIH 2013). The water was tested for the content of microorganisms, and the result was <1 CFU/mL or 0 CFU/mL.

From overnight growth of the culture a broth with turbidity of 0.5 McFarland was prepared, from which by tenfold dilutions a dilution of 10^5 CFU/mL was obtained. 5 mL of broth from 10^5 CFU/mL was added to 5 L of sterilized purified water, which resulted in water contaminated with *E. coli* with a density of 100 CFU/mL. The microbiological load is chosen so that the counts of the grown colonies of microorganisms can be made without difficulty and even slight fluctuations in their quantity can be detected.

That water was filtered through a filter with Ag nanoparticles and without Ag nanoparticles. The entire filtrate was collected in a sterile container. Then, 1 mL of water before and after filtration through filters (with and without Ag) was tested by pour plate method using nonselective nutrient medium (Nutrient agar, Liofilchem, Italy). From each sample, inoculums were tested in six parallel plates, which then were incubated at 37°C for 24–48 h. Then the number of colonies was counted. The experiments were performed in triple replicate for each strain of bacteria separately. The results were averaged, and the degree of antibacterial properties of the filter with Ag nanoparticles and without Ag nanoparticles towards *E. coli* and *E. faecalis* were calculated.

Antibacterial properties of the filter towards mixed culture of microorganisms

The purpose of this experimental series was to study the antibacterial effect of filter cartridge with Ag nanoparticles (0.3 g) against a mixed culture of microorganisms and the changes in its antibacterial activity during filtration of large volumes of highly contaminated water. For this purpose, a structure able to filter large volumes of water with high microbial load was built.

To achieve this, a container with 3-ton holding capacity was filled with water contaminated by soil microorganisms, as one of the sources of water pollution is the soil (Moss 2008). At the outlet of the water container, we assembled two separate pipes and on each one we mounted a filter flask with similar filter cartridges. One of the cartridges contained Ag nanoparticles (0.3 g), the other did not. Before each filter a counter for tracing the volumes of filtered water was mounted. Samplers were installed before and after the filter flasks. Schematic depiction of the structure is shown in Figure 3.

The contaminated water was pumped through both filters (one with Ag, the other without) at the same time. By tracing the filtered water volume, water samples were taken before and after filtration of 100 L, 500 L, and 1,000 L of water. 1 mL of each sample was inoculated into nonselective nutrient medium (Nutrient agar, Liofilchem, Italy). From the sanitary and epidemiological point of view the greatest interest is the mesophilic microorganisms in water, whose optimum growth temperature is 35–37°C, so all plates were incubated at 37°C for 24–48 h. After incubation, the colonies were counted. Based on these data the antibacterial effect of filters with Ag and without Ag was calculated, after filtering different volumes of water.

RESULTS

Antibacterial properties of the filters with Ag nanoparticles

The filtration of water contaminated with *E. coli* ATCC25922 through the filter containing 0.1 g Ag, had

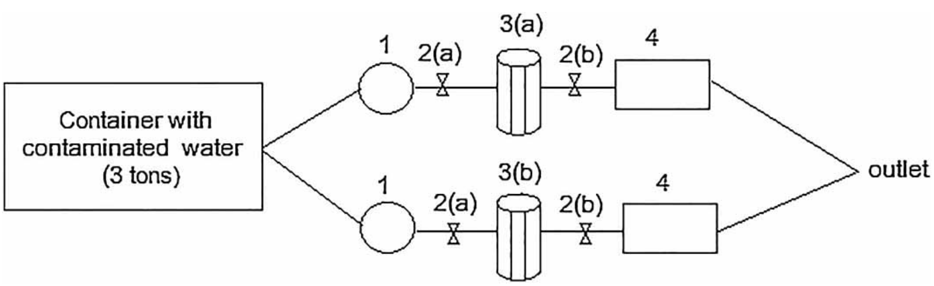

Figure 3 | Scheme of water filtration construction. 1, water meter; 2, samplers: 2(a), before filtration, 2(b), after filtration; 3, flasks with filter cartridges: 3(a), filter cartridge without Ag, 3(b), filter cartridge with Ag; 4, vacuum pumps with flow rate max 35 L/min.

average filtration efficiency of 97.09%. In the case of *E. faecalis* ATCC29212 antibacterial effect of the filter with 0.1 g Ag dropped to 33.34%. When the amount of Ag nanoparticles in the filter cartridge was increased three-fold, the antibacterial effect of nanocomposite filter against *E. faecalis* reached 92.18% (Figure 4).

When water contaminated with *E. coli* was filtered through a filter without Ag nanoparticles, the microorganism retention was 6.4%, and when filtrated with water contaminated with *E. faecalis*, the retention was 4.3% (Table 1).

Since we have only decimal quantitative fluctuations in our experiment, the results are calculated in percentages, not in logarithms, which is more convenient for expressing the reduction of the number of microorganisms by hundreds, thousands, or more times.

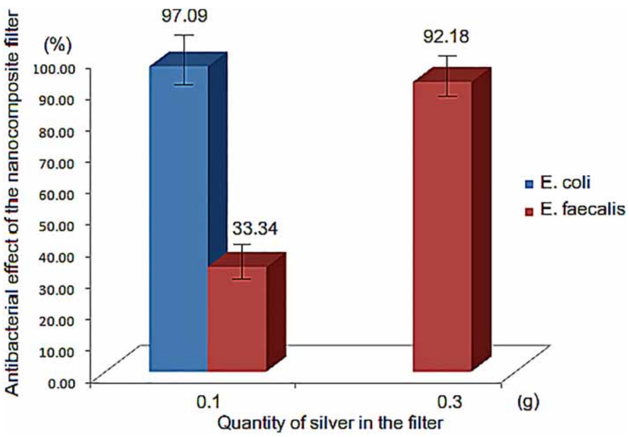

Figure 4 | The antibacterial effect of the filter with different contents of Ag nanoparticles against *E. coli* and *E. faecalis*.

Antibacterial properties of the filter towards mixed culture of microorganisms

After filtration of 100 L of water with a microbial load of 3,940 CFU/mL, the antibacterial effect of the filter without Ag was 12.94% and antibacterial effect of the filter with Ag was 53.30%. After filtration of 500 L of water with a microbial load of 3,720 CFU/mL, the antibacterial effect of the filter without Ag was 9.68% and antibacterial effect of the filter with Ag was 44.35%. Finally, after filtration of 1,000 L of water with a microbial load of 4,300 CFU/mL, the antibacterial effect of the filter without Ag was 15.58% and antibacterial effect of the filter with Ag was 47.91%. Averaging the results, it transpired that after filtration of 1,000 L of highly contaminated water with a microbiological load on average of 3,987 CFU/mL, the antibacterial effect of the filter without Ag totaled 12.73% and the antibacterial effect of the filter with Ag totaled 48.52% (Figure 5).

DISCUSSION

The data show that the same amount of Ag nanoparticles inhibited the growth of various microorganisms, in this case of Gram-negative and Gram-positive bacteria, to varying degrees. Thus, with equal content of Ag in nanocomposite filters the antimicrobial effect against *E. coli* (Gram-negative bacteria) exceeded the antimicrobial effect against *E. faecalis* (Gram-positive bacteria) by 63.75%.

It is known that Gram-negative bacteria are more susceptible to the toxic effect of Ag, as compared with

Table 1 | The results of counting the colonies before and after filtration of water contaminated with *E. coli* ATCC25922 and *E. faecalis* ATCC29212 through the filter with Ag and without Ag

	***E. coli* ATCC 25922**				***E. faecalis* ATCC 29212**			
	Filter with 0.1 g Ag		**Filter without Ag**		**Filter with 0.3 g Ag**		**Filter without Ag**	
	Before filtration	**After filtration**	**Before filtration**	**After filtration**	**Before filtration**	**After filtration**	**Before filtration**	**After filtration**
Mean values with standard errors[a], CFU/mL	122 ± 9	4 ± 2	120 ± 8	112 ± 6	112 ± 2	9 ± 1	116 ± 4	111 ± 4
%	100	2.9	100	93.6	100	7.8	100	95.7
Filterability, %	97.1 ± 1.9		6.4 ± 0.6		92.2 ± 0.7		4.3 ± 0.8	

[a]Mean data and standard errors were from at least three independent experiments.

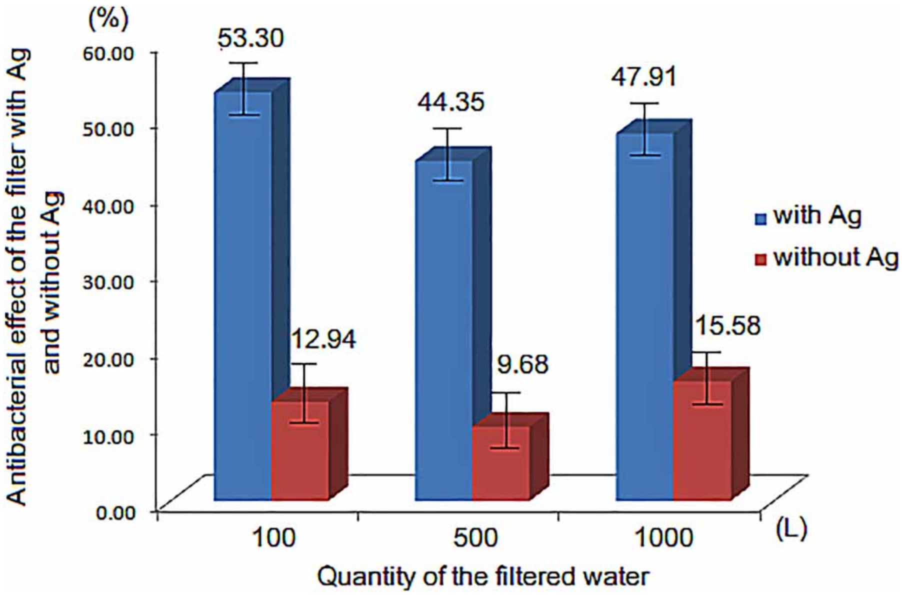

Figure 5 | Antibacterial effect of the filter without Ag and with Ag towards a mixed culture of microorganisms after filtration of 100 L, 500 L, and 1,000 L of water.

Gram-positive bacteria and one reason for that is the difference between the structure of the cell wall (Jung *et al.* 2008). However, with increasing concentration of Ag in the filter, the antibacterial effect of the nanocomposite filter against *E. faecalis* can be enhanced. The difference of the antimicrobial effect of the filter relative to the *E. faecalis* caused by increasing the amount of the Ag by three times is 58.84% (Figure 4). Thus, the results also show that different amounts of Ag nanoparticles cause different effects on the same microorganism, i.e., the strength of the antibacterial effect of Ag depends on its quantity (Kim *et al.* 2007). This implies that the higher Ag concentration could increase antimicrobial activity of the filter cartridge in order to meet the requirements of water quality (European Commission 1998), which is an issue for further research. However, we must take into account that the accumulation of Ag in the human body in excessive amounts can cause a specific disease called 'argyria' or 'argyrosis', which results in pigmentation of the skin, mucous membranes, eyes, and sometimes hair. However, it has been proved experimentally that Ag ions have a bacteriostatic effect, but mutagenic activity has not been revealed, also as a carcinogenic effect (US Public Health Service 1990). In this regard, the study of the strength of interaction of Ag nanoparticles with tuff mineral, depending on the amount of Ag nanoparticles in the cartridge and on the volumes of filtered water, remains a challenge for further research.

In addition, the antibacterial effect of the filter was directly dependent on the bacterial load of the water being filtered. When the average microbial load of the water was approximately 100 CFU/mL, filtration efficiency of the nanocomposite filter containing 0.3 g Ag nanoparticles with respect to *E. faecalis* was 92.2%. By increasing the microbial load to 3,987 CFU/mL, filtration efficiency of the nanocomposite filter containing the same amount of Ag nanoparticles, against mixed culture, dropped to 48.52% (see Figure 5).

Moreover, the antimicrobial effect of the nanocomposite filter with Ag was indirectly influenced by the total and organic pollution of the filterable water, so the permeability of the filter was sensitive to high organic contamination, and thereby the contact area between the water and the filtering material containing Ag nanoparticles was reduced. Thus, the antimicrobial effect of the nanocomposite filter with Ag nanoparticles depended on the mechanical properties of the filter; this means that the antimicrobial activity of the nanocomposite filter with Ag will continue until the physical and mechanical properties of the filter are exhausted.

Therefore, the antibacterial effect of the nanocomposite filter is mainly dependent on the amount of Ag, on the quantity and quality of the microbiological load of the water, and on the total and organic pollution of water.

Among the theories to explain the mechanism of Ag action on microorganisms, the most common is the adsorption theory, according to which the cell loses viability due to interaction between electrostatic forces generated between negatively charged bacterial cell and positively charged Ag ions during their adsorption by bacterial cell (Prabhu & Poulose 2012; Abbaszadegan *et al.* 2015). The cell remains viable, but some of its functions, such as division, are disrupted (bacteriostatic effect). As soon as Ag is absorbed by the cell surface, it penetrates into the cell and inhibits the enzymes of the respiratory chain and uncouples the processes of substances oxidation and oxidative phosphorylation in the microbial cell, eliciting cell death (bactericidal effect).

There are many filters that have antibacterial properties due to the presence of Ag in their composition, e.g., paper (Dankovich & Gray 2011), carbon (Bell 1991), ceramic filters (Kallman *et al.* 2011; van der Laan *et al.* 2014) impregnated with Ag nanoparticles, and so on. Compared with them, the technology in preparation of the filter made from tuff mineral with Ag nanoparticles does not require high costs, much energy for preparation, and has high productivity. The nanocomposite filter also has high strength and long-term operation, since it is known that tuff is a porous mineral, which has strength, ease of processing, and has a very small specific gravity. Due to its structure, it has porosity and has sorption properties, thus providing water purification from high turbidity, color, and iron. In combination with Ag nanoparticles, the antibacterial properties of the filter are provided. Antibacterial activity of the filter also will prevent the possibility of the growth of microorganisms inside the filter cartridges. The filter itself will not become a source of secondary water pollution. Nevertheless, the life of the filter depends on the level of contamination of the filtered water (mechanical particles), which leads to clogging of the cartridge pores. However, it is possible to extend the life of the filter by simple mechanical cleaning of the cartridge. Despite this, there are many aspects to investigate for this filter, which remain a topic for further research.

CONCLUSIONS

Based on the results obtained, we could conclude that the nanocomposite filter with Ag nanoparticles which were adsorbed and deposited on the mineral granules of tuff had an antibacterial effect. It revealed the possibility of providing antibacterial activity to material such as tuff, thereby expanding the range of its application and opening the way for experimentation to obtain materials with antibacterial properties based on other minerals and Ag, which could find practical applications.

It is suggested that the combination of Ag nanoparticles with porous mineral tuff led to producing a highly effective nanocomposite material that can be used for disinfection of drinking water.

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