

PREPARATION OF SILVER NANOPARTICLES – NATURAL ZEOLITE COMPOSITE AND STUDY OF ITS ANTIBACTERIAL PROPERTIES

O. Ts. Gemishev¹, M. I. Panayotova², N. A. Mirdzveli³, V. T. Panayotov⁴

¹Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

²Department of Chemistry, University of Mining and Geology, Sofia, Bulgaria

³Petre Melikishvili Institute of Physical and Organic Chemistry of Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia

⁴Bulgarian Academy of Sciences, Sofia, Bulgaria

Abstract

Composite consisting of natural zeolite and silver nanoparticles (AgNPs-zeolite) has been synthesized by ion exchange followed by a thermal treatment. The prepared material is characterized by BET, EDX, TEM and XPS. The antibacterial activity of the synthesized material has been studied against E. coli. Results on the influence of the material's dose and treatment time on the antimicrobial action of the composite are presented. The minimum inhibitory concentration is 10 mg AgNPs-zeolite/mL of culture medium containing E. coli (10⁵ CFU/mL). Bactericidal activity has been observed after 300 minutes of treatment. The bactericidal action of the synthesized nanocomposite against E. coli is maintained after three repeated re-inoculations, and after the third inoculation it is 99.9%. Possible mechanism of the observed antimicrobial action is briefly discussed.

Keywords: silver nanoparticles zeolite nanocomposite, antibacterial activity, *Escherichia coli*

1. INTRODUCTION AND TASK JUSTIFICATION

Availability of safe water for drinking, household, industrial and recreational purposes represents one of the most serious health and economics problems. It is estimated that 3.6 billion people live in areas that are potentially water-scarce at least one month per year, and this population could increase to 4.8–5.7 billion by 2050 [1]. Health risks may arise from consumption of water contaminated with many different pollutants, among them microbial pathogens. As reported by World Health Organization (WHO), in 2017, 2.2 billion people worldwide used drinking water without safely managed services. WHO estimation is that the 829 000 people die each year from diarrhea as a result of use of unsafe drinking water [2].

Disinfection is of undoubting importance in the supply of safe drinking water. The destruction of pathogenic microorganisms is absolutely necessary. Chlorination is considered to be the most important process for preventing the spread of waterborne diseases. Residual disinfection is used to provide a partial safeguard against low-level contamination and growth within the distribution systems. However, there are some concerns regarding chlorine usage that may inspect its uses, such as: i) Chlorine reacts with many naturally occurring organic and inorganic compounds in water to produce undesirable disinfection by-products (DBPs); ii) Hazards associated with chlorine use require special treatment and response programs; iii) High chlorine doses can cause taste and odor problems.

Disinfection by ozonation and use of ultraviolet light can be also applied but these technologies are more expensive and in addition, they do not possess residual effect, which means that after their application a certain level of chlorination has to be applied to avoid undesirable bacterial growth in distribution systems for already treated water.

The membrane processes of most significance in water disinfection are reverse osmosis, ultrafiltration and microfiltration. However, these technologies are energy and operationally intensive. These systems still require considerable capital investments, engineering expertise and infrastructure.

Development of more easy to use disinfectant that can be applied, for example, in conventional filtration systems represents a challenge.

Silver (Ag) has been known to have antibacterial properties since Roman times. It possesses potential to be a water disinfectant that does not result in DBPs [3]. Effectiveness of silver ion (Ag^+) (generated from silver salts [AgNO_3 , AgCl] or produced electrolytically) was tested against a range of bacteria and its activity was assessed by the log reduction in bacterial numbers.

Hwang et al. [4] studied the efficacy of Ag^+ ions (up to 100 $\mu\text{g/L}$), derived from silver nitrate (AgNO_3), against *Legionella pneumophila*, *Pseudomonas aeruginosa* and *Escherichia coli* (all at 1.5×10^7 CFU/mL) in synthetic drinking water (pH 7, temperature 25 °C). After a three hour contact time with the highest concentration of silver the following log reductions were obtained: *L. pneumophila* - 2.4 log reduction, *P. aeruginosa*- 4 log reduction and *E. coli* - 7 log reduction.

Huang et al. [5] studied the efficacy of Ag^+ ions, derived from AgCl , against 3×10^6 CFU/mL of *P. aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii*. At the highest concentration used (80 $\mu\text{g Ag}^+/\text{L}$) a 5 log reduction in *P. aeruginosa* CFU was seen after 12 hours, while for *S. maltophilia* it was seen after 6 hours. For *A. baumannii* the 5 log reduction was achieved after 72 hours of exposure.

Cunningham et al. [6] carried out experiments aimed at determining the minimum inhibitory concentration (MIC) of AgNO_3 on *Escherichia coli* (*E. coli*). They reported 24 hour MIC of between 60 and 80 $\mu\text{g Ag}^+/\text{L}$. Approximately a 4 log reduction was seen at 100 $\mu\text{g/L}$ after 24 hours of exposure.

Pathak and Gopal [7] evaluated the influence of the concentration of Ag^+ ions (1, 2, 5, 10 and 20 $\mu\text{g/L}$), produced from silver electrodes, for up to 60 minutes on the efficacy of antibacterial activity against *E. coli* (with initial concentration 1.75×10^3 CFU /mL). Complete stopping of the bacteria activity was observed at neutral pH and 20 °C in 20 minutes at 20 $\mu\text{g/L}$ concentration, in 40 minutes - at 10 $\mu\text{g/L}$, in 50 minutes - at 5 $\mu\text{g/L}$, in 60 minutes - at 2 $\mu\text{g/L}$.

It is accepted by the scientific community that when Ag^+ ions are loaded onto a solid carrier, they can be slowly released into the environment, thus their antibacterial activity is maintained over a long period of time [8, 9]. Because of this, silver loaded systems have been used in biomedical applications, for example, as a coating. In this line, different studies have considered the impact of silver coating on ceramic filters.

Zeolites have been also studied as solid carriers of Ag^+ ions. Zeolites type X and A, with different Al/Si ratio were exchanged with Ag^+ and the obtained Ag^+ contents were between 31.4 and 42.7 wt. % [9]. The release of metal ions from the loaded zeolites into tryptic soy broth was from 22.9 - 45.7 mg/L for 0.5 h to 58.1 - 70.8 mg/L for 24 h. At an initial bacteria concentration of 1×10^7 CFU/mL the MIC towards *E. Coli* was 64 $\mu\text{g Ag}^+/\text{mL}$ except for the Ag^+ exchanged zeolite with the lowest Si/Al ratio where the MIC was 32 $\mu\text{g/mL}$.

Silver-exchanged zeolite NaY with different concentrations of uploaded Ag^+ was prepared by zeolite contacting with AgNO_3 with different concentrations based on 25, 50, 100 and 200% of the cation exchange capacity (CEC) of the zeolite [10]. The obtained silver-exchanged zeolite NaY (ZSA) was functionalized by contacting with different concentrations (0.01, 0.2 and 0.4 M) of 3-aminopropyltriethoxysilane (APTES) [$\text{H}_2\text{N}(\text{CH}_2)_3\text{Si}(\text{OC}_2\text{H}_5)_3$]. The disc diffusion test and MIC technique were used to study the antibacterial activity of the prepared materials. The tests showed the highest antibacterial activity of samples prepared by exchange with AgNO_3 in concentrations equal and higher than 50% of the CEC and APTES - 0.01 M. The MIC value with respect to *E. Coli* ATCC11229 was 0.05 g zeolite/L in distilled water and 2 g zeolite/L - for 0.9 % saline solution.

Nowadays, silver nanoparticles (AgNPs) are widely examined as an alternative antibacterial agent to Ag^+ , since it is expected that the high specific surface area of AgNPs considerably increases the area available for the microbes to be exposed to. This allows enhanced interactions with the cell membrane and easy penetration into the cell, leading to more complete destruction of microbial cells compared to the case where Ag^+ ions are used. In addition, when used in reasonable amounts, silver metal does not

negatively affect the human body [3, 11, 12]. By using the InQ™ Cell Research System instrument with HEK 293 cells, it was found that AgNPs at concentration of 2 ppm and 4 ppm were not toxic for human healthy cells, but inhibit bacterial growth [13].

It has been found that the antimicrobial activity of Ag and its compounds is directly proportional to the number of biologically active Ag⁺ ions released, and its availability for interaction with the bacterial cell wall [7, 12]. In this line, AgNPs are a good and more permanent (compared to added Ag⁺ solutions) source of the antimicrobial agent [12]. In order to be applied effectively for water disinfection, nanoparticles must be prevented from aggregation and supported on different substrates.

Lv and coauthors uniformly decorated AgNPs onto porous ceramic materials to form composite by using 3-aminopropyltriethoxysilane (APTES) as a connecting molecule [14]. The composite did not lose AgNPs under washing and was able to be stored for long periods. The composite sterilized water, polluted with *E. coli* (10⁵ CFU/mL) when used as antibacterial filter at a flow rate of 0.01 L/min. However measurements on silver concentration in the filtered water were not reported.

Nangmenyi et al. [15] impregnated fibreglass with different amounts of AgNPs (<30 nm) and studied the performance of thus prepared materials at immersion and during filtration. When silver impregnated mat (1 wt.% Ag) was immersed into 100 ml *E. coli* suspension (10⁶ CFU/mL), *E. coli* was not detected in the suspension after an hour. When *E. coli* concentration of 10¹² CFU/mL was used, the AgNP fibreglass mat (1.8 wt.% Ag) ensured a 7 log reduction in concentration in five minutes. Antibacterial filters (5 wt.% Ag) were prepared and a bacterial solution (10⁶ CFU/mL *E. coli*) was passed through the filter at a flow rate of 20 mL/min. *E. coli* were not found in the treated water (a 6 log reduction, of which 1 log reduction was due to the fibreglass).

Heidarpour et al. [16] studied the capability of AgNPs-coated polypropylene filters to remove *E. coli* from water. They passed 15 litres of distilled water containing 10³ CFU/mL *E. coli* through silver-coated filters or uncoated at a flow rate of 3 L/h. The *E. coli* concentration from the silver-treated filter was zero after 7 hours of filtration and recirculation, whereas the concentration in the water from the untreated filter remained at 10³ CFU/mL. Scanning electron micrographs showed that *E. coli* cells were attached to the surface of the AgNPs coated filter. AgNPs were not found in the treated water. The observed bacterial removal was assigned to a combination of the bacteriostatic and bactericidal impact of the silver and the decrease in the pore size of the Ag-treated polypropylene (pore size of 1.3 µm in comparison with the untreated material - 9.9 µm).

Zeolites have been also considered a promising inorganic material for hosting AgNPs. Because of their rigid and stable network of hollow channels and pores and their thermal stability, natural and synthetic zeolites are ideal templates for formation and growth of various nanoparticles. Even more, small nanoparticles are physically prevented from aggregation to larger nanoparticles or micron-sized particles [17]. Thus, the zeolites have attracted the attention as promising substrate for producing composite materials bearing AgNPs.

Flores-Lopez and coauthors [18] prepared composite representing AgNPs supported on the surfaces of the natural zeolite chabazite. The bactericide properties of the prepared material (containing 18.57 % Ag) were tested against several types of bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella flexneri* and *Pseudomonas aeruginosa*). A saline solution containing powdered silver chabazite nanocomposite at different concentrations, including a control sample of chabazite without silver, was mixed with the bacteria (10⁵ CFU/mL) and incubated for 1, 24 and 48 h. After incubation time, the number of bacteria colonies were counted in each case. It has been found that the killing of bacteria was complete in all cases except for *S. aureus*, where two CFU/ mL were found after 48 h.

Synthetic commercial zeolite A (NaA) was loaded by impregnation with Ag⁺ ions. Further, the Ag⁺ ions were reduced with sodium borohydride to prepare AgNPs loaded NaA [19]. The antibacterial activity of the AgNPs-NaA composites was studied by determining the reduction in the number of viable *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922) bacteria at initial concentration of 10⁷ CFU/mL. The composite showed a dose- and time-dependent antimicrobial

activity against the Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria, but was considerably more effective against *E. coli*.

The WHO report issued in 2018 (but generally covering studies in the period 2005 - 2015) [3] and aimed at assessing the efficacy of application of silver (ionic silver and silver nanoparticles) in drinking water treatment concluded that "the overall evidence base does not indicate that the supplemental use of silver nitrate or silver nanoparticle application in water filters improves the microbiological quality and safety of the filtered water".

However, the demand for safe potable and household water is increasing, as well as the efforts of scientific community in this direction.

A household water treatment filter device has been developed that is fabricated from man-made zeolite cotton packed in a tube [20]. Zeolite cotton (ZC) is elaborated by growing mesoporous zeolite onto the surface of cotton fiber. The material showed cation-exchange capacity over 6.5 mg/g (Cu^{2+}) and treatment efficiency of 84 mL/(day g), ensuring the removal of heavy metals to the concentrations less than 5 $\mu\text{g/L}$. In order to give it antibacterial properties, the zeolite cotton is loaded with silver - 1 mg/g. The experiment with bacterial solution having *E. coli* concentration of 0.97×10^6 CFU/100 mL showed that no viable colony was obtained in the solution flowed through a tube containing 5 g of Ag exchanged ZC (AgZC). However, the remained Ag^+ concentration in the effluent was 0.310 ppm, 6 times over the safety limit. Further, a dual-bed filter configuration was proposed in which 5 g of AgZC were set at the top of the filter and 5 g ZC were placed at the bottom. The low mass loading of silver does not significantly affect the performance of ZC for heavy metal ions. The idea is that bacterial polluted water would be initially purified by AgZC. Further, the excessive Ag^+ , possibly released from the AgZC above, would be re-adsorbed by un-exchanged ZC. In this way the water leaving the filter tube would contain neither bacteria nor overdose Ag^+ ions. The concentration of Ag^+ was 0.025 ppm that is lower than the safety concentration.

The above idea may be developed further by using natural zeolite that does not require chemicals and energy for its synthesis. Incorporation of AgNPs in the zeolite would ensure a more uniform effect, where microorganisms will be more exposed to silver released from the nanoparticles.

In this regard, and on the basis of our preliminary experiments [21, 22], we have decided to continue the work on the preparation of AgNPs-clinoptilolite nanocomposites, and in particular - on testing their antimicrobial activity, which is the main subject of this article.

2. MATERIALS AND METHODS

2.1. Preparation and characterization of AgNPs-clinoptilolite composite

Natural zeolitic rock from East Rhodopes region (Bulgaria) was used in the study. The material was milled, sieved and the fraction 0.09-0.325 mm was utilized. After its washing and drying, the material was analyzed - classical silicate analysis was applied to determine its chemical composition that was (in wt. %): SiO_2 – 70.28, Al_2O_3 – 10.90, CaO – 2.97, MgO – 0.51, K_2O – 3.41, Na_2O – 0.46, Fe_2O_3 – 0.28, MnO – 0.04, TiO – 0.06, P_2O_5 < 0.05, SO_3 < 0.05, LOI – 10.99. The theoretical cation exchange capacity of the material was 211 meq/100 g zeolite. The XRD analysis of the washed material has shown that it contained 73 % clinoptilolite.

Silver ions were loaded on the zeolite by contacting it with 0.1 M AgNO_3 solution at solid to liquid ratio = 1 : 20 for 4 hours. Silver-loaded zeolite (71 mg Ag/g zeolite) was washed with distilled water until negative reaction for Ag^+ in washings was observed. Then it was dried at 50 °C overnight. The Ag-loaded zeolite was heated at 600 °C under air conditions for 2 hours to obtain AgNPs-clinoptilolite composite (AgNPs-Z). Considering silver light sensitivity, all experiments were carried out under dark conditions.

The specific surface area of AgNPs-composite was measured by applying the Brunauer, Emmett and Teller (BET) method, using Quantachrome NOVA 1200e Analyzer working with N_2 . EDS elemental mapping and EDX spectrum were made with JEOL - JSM-6010PLIS/LA. The XPS measurements

were carried out in the analysis chamber of electron spectrometer Escalab-MkII (VG Scientific) with a base pressure of 1×10^{-10} mbar. The spectra were excited with AlK α radiation ($h\nu = 1486.6$ eV) at instrumental resolution of 1.1 eV as measured by FWHM of Ag3d5/2 photoelectron line. TEM analysis of prepared composite was carried out on JEOL, model JEM 2100, 200 kV analytical electron microscope. Concentration of Ag⁺ ions released in the inoculated culture medium was determined by ICP-OES.

Details on the preparation of the Ag-loaded zeolite and the AgNPs-composite, as well as on composite characterization, can be found in our previous work [21, 23].

2.2. Antibacterial tests

E. coli strain 3398 was pre-grown on a Luria agar for 16 h at 37 ± 0.1 °C to obtain cultures in a log phase of growth. Peptone water (PW) was prepared by dissolving 10.0 g peptone and 0.5 g NaCl in 1 L of distilled water. Samples of 10 mL of peptone water were inoculated with *E. coli* (10^5 CFU/mL). In order to study the minimum inhibitory concentration (MIC) of the synthesized AgNPs-clinoptilolite composite, the synthesized material was added to the inoculated PW in concentrations from 1 to 20 mg/mL. Thus prepared samples were cultivated for 24 hours at 37 ± 0.1 °C. Further, 1 mL of the suspension was serially diluted in triplicate in sterile distilled water and volumes of 0.1 mL were aseptically inoculated onto the Lysogeny agar-Luria (0.5 g/L NaCl) plates. The plates were incubated at 37.0 ± 0.1 °C for 24 h. After the incubation period, the bacterial colonies were counted and the number of viable cells was reported as CFU/mL. The controls were natural zeolite (from 1 to 20 mg/mL suspensions in PW) and *E. coli* suspension in PW but without any addition of zeolite. The controls were treated in the same manner (dilutions and incubation) as the sample variants. Results for MIC determination are expressed as % of bactericidal activity of AgNPs-Z (CFU_{AgNPs-Z}) with respect to untreated control (CFU_{control}), i.e.

$$\% \text{ of bactericidal activity} = [(CFU_{\text{control}} - CFU_{\text{AgNPs-Z}}) / CFU_{\text{control}}] \times 100.$$

In order to establish the minimum exposure time required for the bactericidal activity, 100 mg of composite was added to 10 mL of peptone water inoculated with *E. coli* (10^5 CFU/mL). Samples of 0.1 mL were taken at 1, 5, 30, 60, 180, 300 and 420 min, inoculated onto the Lysogeny agar-Luria (following the same dilutions as above) and incubated at 37.0 ± 0.1 °C for 24 hours. Then, the bacterial colonies were counted and reported as CFU/mL.

Multiple use of bactericide materials is important from both economic and technological point of view. However, such studies are very scarce [24]. In order to study the possible re-use of the synthesized nanocomposite, one and the same material was used three times (in concentrations 10 mg/mL) to impact PW inoculated with *E. coli* (10^5 CFU/mL). After the first and second 7 hours cycle of its use, the nanocomposite was separated and washed with sterile distilled water. Then, the test procedure followed the one with application of virgin nanocomposite. The bactericidal activity of AgNPs-zeolite is presented as % of with respect to untreated control.

3. RESULTS AND DISCUSSION

3.1. AgNPs-composite characterization

BET measurements showed 22% reduction in the specific surface area of AgNPs-composite, compared to unloaded zeolite treated under the same temperature, which confirms the incorporation of AgNPs into the zeolite.

The EDX spectrum of the composite showed the presence of the basic elements found in zeolite, in wt. %: O (47.69), Si (32.52), Al (7.42), K (2.67), and Ca (1.64), as well as Ag (8.06). EDS elemental mapping showed that silver is uniformly distributed in the zeolitic material - Figure 1.

XPS spectra (Ag3d photoelectron and AgMNN Auger-lines) indicated presence of Ag⁰ in the composite.

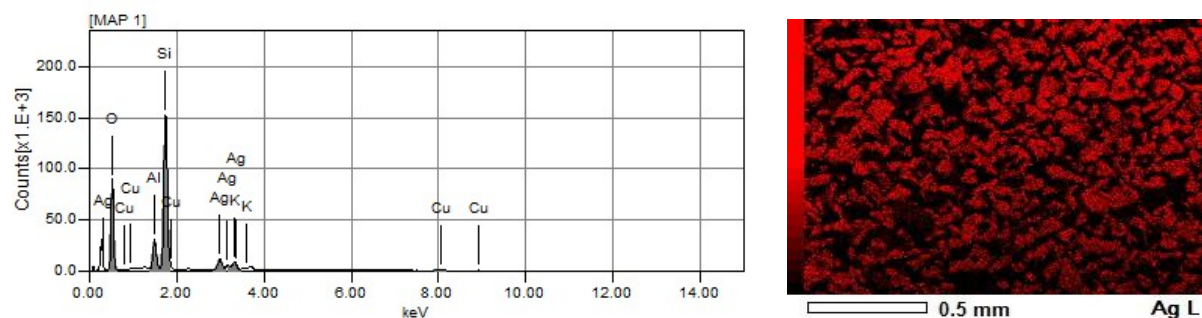


Fig. 1. EDX spectrum and elemental mapping (Ag) of the zeolite applied in the antimicrobial tests

TEM analysis of prepared composite revealed that AgNPs with diameter in the range of 20-30 nm were observed on the material's surface and particles with diameter in the range of 3-6 nm were situated in the zeolite secondary pores - Figure 2.

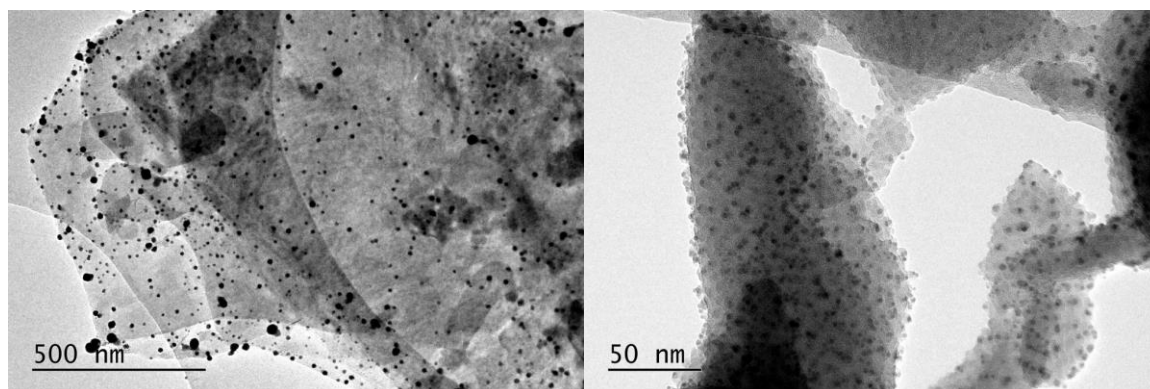


Fig. 2. Transmission electron microscope images of the AgNPs-zeolite composite

3.2. Antibacterial activity of AgNPs-composite

Gram-negative bacterium *E. coli* was used as an indicator strain to screen the antibacterial activity of AgNPs-clinoptilolite composite. The experiments for determining the MIC were performed with AgNPs-Z concentrations from 1 mg/mL to 20 mg/mL, and the results are presented as % bactericidal activity compared to untreated control - Figure 3.

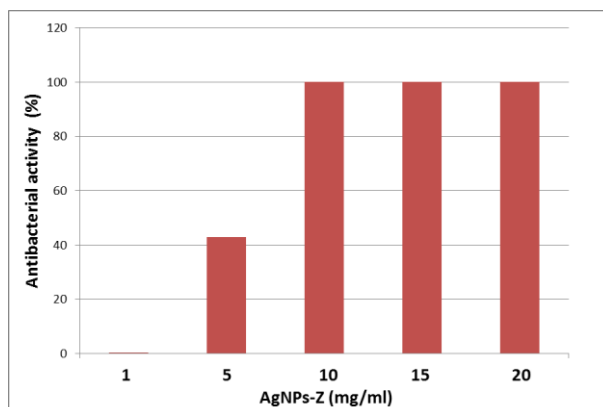


Fig. 3. Antibacterial activity of AgNPs-Z against *E. coli* (%)

Natural zeolite (not bearing AgNPs), applied in concentrations identical to those of the AgNPs-Z nanocomposite, did not show any antimicrobial activity, even some increase in the CFU was observed. An increase in the number of colonies on zeolite without silver was found also by other studies [11]. The finding could be explained by the creation of better conditions for the development of the microorganism by fixing it on the zeolite surface.

As it can be seen from Figure 3, AgNPs-Z composite, when applied in concentration of 1 mg/mL, had no bactericidal effect against *E. coli*. Increasing the applied concentration of the AgNPs-Z nanocomposite lead to appearance of bactericidal activity. The MIC was established at a AgNPs-Z concentration of 10 mg/mL. The MIC value with respect to *E. Coli* found by us is in the range of that found by Hanim et al. [10] in 0.9 % saline solution, i.e. 2 mg zeolite/mL, but in their case the Ag load on zeolite was approximately 4 times higher.

The determined MIC was used to study the antibacterial activity of AgNPs-Z, as a function of the time, of the test microorganism exposure. *E. coli* was treated with 10 mg/mL AgNPs-Z for 1, 5, 30, 60, 180, 300 and 420 min and the number of viable cells (CFU/mL) was counted. Data obtained are presented in Figure 4.

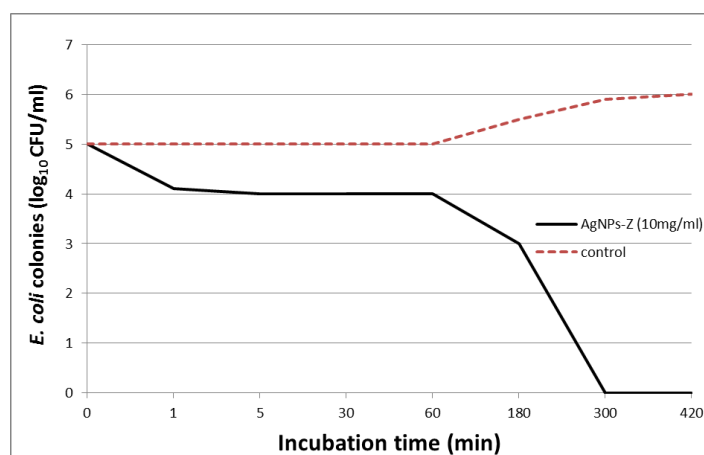


Fig. 4. Evolution of number of *E. coli* colonies survived in presence of 10 mg/mL AgNPs-Z, as a function of time

As it can be seen in the Figure 4, the control (*E. coli* suspension in PW without any zeolite) exhibits bacteria growth to 10^6 CFU/mL. The survival of the strain decreased by one degree after 60 minutes of exposure - Figure 4. The viability of the test microorganism dropped sharply after the treatment of 180 min. Cell growth was completely absent after 300 min of contact. Our results are comparable to the results of Guerra et al. [11] for AgNPs - Mexican clinoptilolite composite, who observed a decrease in the number of colonies (when contacted with 3.3 mg/mL composite containing 2.1 wt. % of silver) after 60 min and all bacteria killing after 120 min, but for initial concentration of 2.5×10^2 CFU/mL. Flores-Lopez and coauthors [18] also found 60 min as enough time for killing 10^5 CFU/mL *E. coli* when contacting with 1 wt. % of composite representing silver nanoparticles supported on the surfaces of the natural zeolite chabazite, but their chabazite-based composite was approximately 2.5 times richer in silver.

We extended the contact of *E. coli* with the composite other 120 min (totally 420 minutes) to confirm the bactericidal (not only bacteriostatic) action of the material - practically all bacteria were killed till 7th hour. The bactericidal concentration of 10 mg/mL AgNPs-Z (corresponding to 710 μ g bound silver per mL) determined by us is near to the reported for commercial 4 Å zeolite loaded with 56 mg silver per g of zeolite [25]. The lasting effect could be explained by the slow-release theory, according which Ag^+ could be slowly released from AgNPs-Z into the medium containing *E. Coli*. Actually, many authors relate the antibacterial activity of AgNPs or AgNPs-composites with the amount of the

released Ag^+ ions [12, 26]. It has been reported that 100 $\mu\text{g Ag}^+$ per L of water kill around 60 % of different bacteria and 100 % are killed by 200 $\mu\text{g Ag}^+/\text{L}$ [26]. We have to note that the later value is equal to the USEPA health advisory concentration for drinking water [3]. It is assumed that the released Ag^+ ions react with sulphydryl ($-\text{SH}$) groups of bacteria thus destroying the enzyme activity of bacterial cells. As a result the cells lose their division and proliferation abilities and die [25].

The possibility for a repeated use of the AgNPs-Z composite is important for its practical application. Results from the triple application of one and the same material (after its separation and washing) at concentration 10 mg/mL are presented in Figure 5.

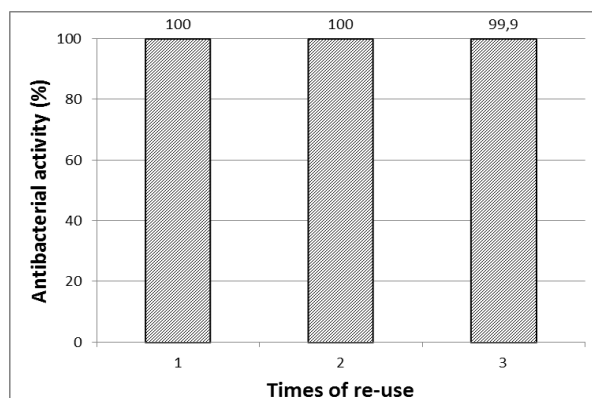


Fig. 5. Antibacterial activity of AgNPs-Z (10 mg/mL) after three times of re-use towards *E. coli*

As it can be seen from Figure 5, the nanocomposite contacting with *E. coli* exhibits 100% antibacterial activity in the initial 2 cycles of use and in the third cycle the activity is 99.9%. The persistent bactericidal action supports the notion of a slow continuous release of Ag^+ from the composite. The zeolite matrix in which AgNPs are embedded ensures AgNPs homogeneous distribution along the surface, thus enabling uninterrupted release of Ag^+ and preventing nanoparticles' agglomeration.

The idea for Ag^+ continuous release is supported also by the residual silver concentrations found - 1.75, 1.45, 1.05 mg/L - after 1st, 2nd and 3rd application cycle correspondingly. These concentrations are higher than the health advisory concentration for drinking water, but significantly lower than the concentration of silver ions that would be obtained if all the silver present in the composite is leached for a given stage. Our previous experiments [23] showed that in the low concentration range natural zeolite immobilizes over 90% of Ag^+ from water solutions. Consequently, contacting the disinfected water with natural clinoptilolite is expected to remove the residual Ag^+ ions and the use of the combination AgNPs-Z / natural zeolite is foreseen as the future development of the study.

4. CONCLUSIONS

AgNPs-zeolite composite has been successfully prepared by natural clinoptilolite loading with Ag^+ ions and its reduction at 600 °C under air conditions. The composite contains small (with diameter 3–6 nm) AgNPs located in the zeolite secondary pores, and bigger AgNPs (with diameter 20–30 nm) that are located on the surface of the material.

The AgNPs-Z composite shows significant bactericidal action against Gram-negative bacterium *E. coli*. The minimum inhibitory concentration is 10 mg AgNPs-zeolite/mL for culture medium containing *E. coli* (10^5 CFU/mL). Bactericidal activity has been observed after 300 minutes of incubation.

The bactericidal action of the prepared nanomaterial against *E. coli* is maintained after three consecutive re-inoculations, and after the third inoculation it is 99.9%.

ACKNOWLEDGEMENTS

The authors thank the National Science Fund of Bulgaria for the financial support of the project DN-17/20.

REFERENCES

1. United Nations World Water Assessment Programme 2018, *The United Nations World Water Development Report 2018: Nature-Based Solutions for Water*, UNESCO, Paris.
2. World Health Organization, *Drinking water - key facts*, viewed 17 May 2020, <<https://www.who.int/en/news-room/fact-sheets/detail/drinking-water>>.
3. World Health Organization 2018, *Alternative drinking-water disinfectants: bromine, iodine and silver*, CC BY-NC-SA 3.0 IGO, Geneva.
4. Hwang, MG, Katayama, H & Ohgaki, S 2007, 'Inactivation of *Legionella pneumophila* and *Pseudomonas aeruginosa*: Evaluation of the bactericidal ability of silver cations', *Water Research*, vol. 41, pp. 4097-4104.
5. Huang, HI, Shih, HY, Lee, CM, Yang, TC, Lay, JJ & Lin, Y 2008, 'In vitro efficacy of copper and silver ions in eradicating *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii*: Implications for on-site disinfection for hospital infection control', *Water Research*, vol. 42, pp. 73-80.
6. Cunningham, JH, Cunningham, C, van Aken, B & Lin, LS 2008, 'Feasibility of disinfection kinetics and minimum inhibitory concentration determination on bacterial cultures using flow cytometry', *Water Science and Technology*, vol. 55, pp. 937-944.
7. Pathak, SP & Gopal, K 2012, 'Evaluation of bactericidal efficacy of silver ions on *Escherichia coli* for drinking-water disinfection', *Environmental Science and Pollution Research*, vol. 19, pp. 2285-2290.
8. Ferreira, L., Fonseca, AM, Botelho, G, Almeida-Aguiar, C & Neves, IC 2012, Antimicrobial activity of faujasite zeolites doped with silver, *Microporous and Mesoporous Materials*, vol. 160 pp. 126–132.
9. Demirci, S, Ustaoglu, Z, Yilmazer, GA, Sahin, F & Baç, N 2014, 'Antimicrobial properties of zeolite-X and zeolite-A ion-exchanged with silver, copper, and zinc against a broad range of microorganisms', *Applied Biochemistry and Biotechnology Part A: Enzyme Engineering and Biotechnology*, vol. 172, pp. 1652-62.
10. Hanim, SAM, Malek, NANN & Ibrahim, Z 2016, 'Amine-functionalized, silver-exchanged zeolite NaY: Preparation, characterization and antibacterial activity', *Applied Surface Science*, vol. 360, pp. 121–130.
11. Guerra, R, Lima, E, Viniegra, M., Guzman, A & Lara, V 2012, 'Growth of *Escherichia coli* and *Salmonella typhi* inhibited by fractal silver nanoparticles supported on zeolites', *Microporous and Mesoporous Materials*, vol. 147, pp. 267–273.
12. Rudramurthy, GR, Swamy, MK, Sinniah, UR & Ghasemzadeh, A 2016, 'Nanoparticles: alternatives against drug-resistant pathogenic microbes', *Molecules*, vol. 21, 836.
13. Okafor, F, Janen, A, Kukhtareva, T, Edwards, V & Curley, M 2013, 'Green synthesis of silver nanoparticles, their characterization, application and antibacterial activity', *International Journal of Environmental Research and Public Health*, vol. 10, pp. 5221-5238.
14. Lv, Y, Liu, H, Wang, Z, Liu, S, Hao, L, Sang, Y, Liu, D, Wang, J, Boughton, RI & Membr J. 2009, 'Silver nanoparticle decorated porous ceramic composite for water treatment', *Journal of Membrane Science*, vol. 331: pp.50-57.

15. Nangmenyi, G, Xiao, W, Mehrabi, S, Mintz E, Economy, J 2009, 'Bactericidal activity of Ag nanoparticles impregnated fibreglass for water disinfection', *Journal of Water and Health*, vol.7, no.4, pp. 657-663.
16. Heidarpour, F, Ghani, WA, Fakhru'l-Razi, A, Sobri, S, Heydarpour, V, Zargar, M & Mozafari MR 2011, 'Complete removal of pathogenic bacteria from drinking-water using nano silver-coated cylindrical polypropylene filters', *Clean Technology and Environmental Policy*, vol.13, pp. 499-507.
17. Yee, MSL, Khiew, PS, Tan, YF, Chiu, WS., Kok, YY & Leong, CO 2015, 'Low temperature, rapid solution growth of antifouling silver-zeolite nanocomposite clusters', *Microporous and Mesoporous materials*, vol. 218, pp. 69-78.
18. Flores-Lopez, NS, Castro-Rosas, J, Ramirez-Bon, R, Mendoza-Cordova, A, Larios-Rodriguez, E & Flores-Acosta M, 2012, 'Synthesis and properties of crystalline silver nanoparticles supported in natural zeolite chabazite', *Journal of Molecular Structure*, vol. 1028, pp.110–115.
19. Jiraroj, D, Tungasmita, S & Tungasmita, DN 2014, 'Silver ions and silver nanoparticles in zeolite A composites for antibacterial activity', *Powder Technology*, vol. 264, pp. 418–422.
20. Chen, X, Yu, L, Zou, S, Xiao, L & Fan, J 2020, 'Zeolite cotton in tube: A simple robust household water treatment filter for heavy metal removal', *Scientific Reports*, vol. 10, 4719.
21. Panayotova, MI, Mintcheva, NN, Gemishev, OT, Tyuliev, GT, Gicheva, GD & Djerahov, LP, 2018, 'Preparation and antimicrobial properties of silver nanoparticles supported by natural zeolite clinoptilolite', *Bulgarian Chemical Communications*, vol. 50F, pp. 211–218.
22. Dolaberidze, NM, Tsitsishvili, VG, Khutsishvili, BT, Mirdzveli, NA, Nijaradze, MO, Amiridze, ZG & Burjanadze, MN 2018, 'Silver- and zinc-containing bactericidal phillipsites', *New Materials, Compounds and Applications*, vol. 2, no. 3, pp. 247-260.
23. Panayotova, M, Mintcheva, N, Djerahov, L & Gicheva, G 2018, 'Kinetics and thermodynamics of silver ion immobilisation by natural clinoptilolite', *Journal of Mining and Geological Sciences*, vol. 61, part II, pp. 87-91.
24. Acuña, OE, Meza-Villezcás, A, Vasquez-Peña, M, Raymond-Herrera, O, Villavicencio-Garciã, H, Petranovskii, V, Vazquez-Duhalt, R & Huerta-Saquero, A 2016, 'Synthesis and complete antimicrobial characterization of CEOBACTER, an Ag- based nanocomposite', *PLoS ONE*, vol. 11, no. 11, e0166205.
25. Zhou, Y, Deng, Y, He, P, Dong, F, Xia, Y & He, Y 2014, 'Antibacterial zeolite with a high silver-loading content and excellent antibacterial performance', *The Royal Society of Chemistry Advances*, vol. 4, 5283.
26. Ouay, BL & Stellacci, F 2015, 'Antibacterial activity of silver nanoparticles: A surface science insight', *Nano Today*, vol. 10, pp. 339-354.