Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.26688

213 (2021) 64–74
February

Hybrid water disinfection system with silver ion in continuous flow ultrasonic reactor

Fadime Karaer Özmen[a,*], Ali Savaş Koparal[b]

[a]*Department of Environmental Engineering, Engineering Faculty, Eskisehir Technical University, 26555 Eskisehir, Turkey, email: fadimek@eskisehir.edu.tr*
[b]*Open Education Faculty, Anadolu University, 26555 Eskisehir, Turkey, email: askopara@anadolu.edu.tr*

Received 11 November 2019; Accepted 20 September 2020

ABSTRACT

The studies on alternative disinfection applications have been performed to protect public health from waterborne disasters, have recently accelerated to attain more effective inactivation of resistant microorganisms present in water. Usage of ultrasound (US) and silver ion (Ag^+) are foremost applications among newly developed disinfection alternatives in water treatment even if these applications have some disadvantages. In this presented work, the hybrid application of US and Ag^+ ion was performed in the continuous flow ultrasonic reactor for the disinfection of *Klebsiella pneumoniae* aiming to eliminate disadvantages of these two techniques for the first time in the literature. In this study *K. pneumoniae* (1×10^4 CFU/mL) was disinfected using three different ultrasonic reactors, which were operated with three frequencies (22, 36, and 833 kW) at continuous flow condition (5, 15, and 25 mL/min) applying different power (36, 51, 67, and 95 W). The disinfection findings were performed using three silver ion concentrations (0.1, 0.01, and 0.005 mM Ag^+) whose concentrations were limited with regard to water and health regulations of silver ion. For the *K. pneumoniae,* 4 log inactivation was observed in USR22 with 22 kHz frequencies, 95 W power, and 0.005 mM Ag^+ at 10 min retention time. The US + Ag^+ provided greater microbial inactivation yield in smaller disinfection time than single applications of Ag^+ and reduces the Ag^+ amount used to inactivate the same bacterial concentration.

Keywords: Disinfection; Ultrasound; Silver ion; Continuous flow; *Klebsiella pneumoniae*

1. Introduction

Metal ions can be used to control microorganisms for various purposes, such as water disinfection, antibacterial product development, and nanomaterial with high bactericidal efficiency. Copper, silver, titanium, and cobalt ions have been widely used for microbial protection as an alternative disinfection technology. Metal ions affect various microorganisms when they are applied with water even if they have a concentration lower than 1 ppm because of their oligodynamic effect [1]. This effect is described at microbiology literature to be high microbial inactivation efficiency via using metal ions like in silver ion with low concentration to solve well-known problems associated with disinfection by-products of conventional chemical disinfection methods with enhanced synergistic disinfection activity combination with other disinfection methods [2,3]. When fungi or bacteria absorb silver ions with 10^5–10^7 number of atoms, silver ion inactivate fungi, or bacteria depending on the colony number in the water [4,5]. Silver ion inactivation mechanism is described as DNA damages resulting from cellular ingestion of silver by a bacteria cell wall and binding to proteins resulting in enzyme inactivation and to lipids, interfering with membranes [6].

* Corresponding author.

Abad et al. [7] investigated the inactivation effect of copper (Cu) and silver (Ag) for various viruses in water including a low level of free chlorine, and they attained 4-log reduction using 0.5 mg/L Cu and 1.0 mg/L Ag but the inactivation was reported as limited because of aggregation of viruses. Bao et al. [8] examined the *Escherichia coli* and *Staphylococcus aureus* inactivation using nanocomposite material including silver nanoparticles (AgNPs) and graphene oxide (GO) for the purpose of ceramic membranes development for water filtration [8]. Another study was taken place to support the improvement of the biofouling resistance of ultrafiltration membranes with silver [9]. Zhang and Zhang [10] investigated the effect of various metal oxides, such as TiO_2, NiO, and ZnO on chlorine disinfection and solar disinfection of MS2 bacteriophage of *E. coli*. They found that metal oxide NPs could lead to complex implications on microbial inactivation in the water disinfection processes thanks to their adsorption affinity on microbial cells. Particularly, the studies were performed for ceramic filtration membranes improved by silver NPs (providing 4% Ag release from the filter) in order for disinfection of the water with different quality [11,12]. The most of studies were aimed to reduce the silver release to the water body. Metal oxide NPs and Ag NPs were used for hybrid photoelectrocatalysis providing fast disinfection and high Ag NPs stability on the surface of the electrode [13]. The released silver concentration to water was recovered in the study made by De la Rosa-Gomez et al. [14] with sodium-modified clinoptilolite column (ZSNa) after disinfection of *E. coli* in the silver-modified clinoptilolite–column (ZSAg) with continuous operations. Furthermore, organic pollutant was removed in the polyurethanes filter media doped with Cu and Ag NPs in addition to bacterial inactivation [15]. Though researchers were focused the high technology thin film improved by TiO_2 and Ag NPs used for antibacterial product for the new resistant bacteria against the common antibiotics [16], some of them were examined the effect of the Ag NPs on granular activated carbon adsorption (GAC) as listed the often-preferred advanced treatment [17].

Ag^+ ion has practiced with simultaneous water treatment methods, in particular, photocatalytic applications to reduce total silver amount released into the water with the aim of avoiding environmental risks and health-related problems of its usages. Zhao et al. [18] achieved the 5-log strong bacterial inactivation of *E. coli* with Ag NPs (1 mg/mL) in parallel with the extensive and insistent release of Ag ion inside the bacterial cell with translocation effect of nano-sized feature of Ag NPs combined with UVA. With the same goal, Agnihotri et al. [19] immobilized the Ag NPs on the amine-functionalized silica surface (≈35% Ag w/w) to enhance the bacterial killing and obtain multiple reusage. Sharma et al. [20] improved disinfection efficiency of filter media produced alumina by immobilized metallic Ag ion providing an effective reduction of *E. coli* within 10–15 s disinfection time thanks to the bactericidal and bacteriostatic effect of Ag ion in addition to adsorption onto filter media [20]. In the application studied hydroxyapatite/TiO_2 nano-composites enclosed by silver halides used for photocatalytic disinfection, 7-log *E. coli* reduction was achieved with 12–24 mg nanocomposites in less than 30 min disinfection period [21]. In order to attain adequate bacterial inactivation with a combination of the different type of UV, Hoang et al. [22] reported that the impregnation–derived Ag (21 nm) and gel–derived Ag–TiO_2–SiO_2 (9 nm) nanomaterials was safely used for drinking water disinfection under both UVC application and dark conditions via their synergistic effects. Hybrid photocatalytic applications of Ag ion (1% Ag doped nanoparticles) were also investigated for different bacteria, such as *Pseudomonas aeruginosa* and *Bacillus subtilis* [23]. These studies were performed to cover the disadvantages of UV in turbid waters and to attain high disinfection performance for different microorganisms.

Recent disinfection studies using metal ions have proven that silver ion has a notable microbial effect among various cells, which applies to new bacteria species resistant to antibiotics [3]. Ag ion has also a lower cytotoxic effect on mammalian cells than that of other metal ions [24,25], and Ag ion inactivates various microorganism species, such as bacteria varying from human enteric viruses [1], coliforms to Gram (+) and Gram (–) [2–17], and yeasts [26,27]. Alonso et al. [28] investigated the bactericidal activity of silver (Ag) and cobalt (Co) NPs produced with 0.1 M $Co(NO_3)$ and 0.001 M $AgNO_3$ against *E. coli*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, *S. aureus* with an initial concentration 10^5 CFU/mL, they reported that Ag/Co NPs were effective for these bacteria with a 5-log reduction when they were applied to polymer fiber contain 0.006 mg Ag [28]. Apart from hybrid metal nanocomposites, Ag ion was examined in polyethersulfone (PES) membranes (including 2% w/w Ag), which were used for ultrafiltration due to high mechanical and chemical resistance, in a study performed by Basri et al. [29] for *E. coli* inhibition, and they found that the release Ag concentration was equal to 0.015 ppm from PES membrane doped with 2% Ag. Barani et al. [30] were reported that the minimum inhibition concentration (MIC) was 25 μL and minimum bactericidal concentration (MBC) was 40 μL for *E. coli* when Ag NPs used in phospholipids membrane [30]. In addition to antimicrobial materials produced with micro-sized and nano-sized Ag requiring advanced technology were chosen by the researchers because of lower Ag ion release which was reported about 1%–2% percentage of total Ag amount used in these products via their stabile surfaces. Researchers indicated that these products maintain their antimicrobial activity for a long period of time. Products also can be used repeatedly and economically with decreasing environmental risks [1–17,24–30]. In summary, Ag ions have been widely used in the literature as an alternative disinfection technology for microbial protection, even though their concentrations used for water disinfection are limited in terms of public health.

Ultrasound (US) that inactivates microorganisms using the energy created by the collapse of cavitation is known as the newly developed water disinfection process including hybrid and consecutive applications in literature. Joyce et al. [31] established the ultrasonic disinfection for *B. subtilis* using low-frequency US (20 and 38 kHz) and high-frequency US (512 ang 850 kHz) to determine the effect of frequency, applied power, and disinfection time, and they found that a remarkable enhancement in inactivation for *Bacillus species* was observed with increasing exposure time and intensity using low-frequency US, while a meaningful increase in bacteria count indicating the fragmentation of colonies were obtained using high-frequency US [31]. In other studies, the low-frequency US disinfection was performed for *Legionella pneumophila* and its host *Acanthamoeba castellanii* using 36 kHz, and the researchers stated that 1.3 log bacterial inactivation was observed thanks to powerful mechanical bacterial cell decomposition effect of low-frequency US, and suggested that low-frequency US could be more effective when the amoebae and associated intracellular *Legionella* was not inactivated with chlorination [32]. It was stated that enhancing high bacterial inactivation efficiency requires high power density, and so, the investigations on ultrasonic hybrid and consecutive applications accelerate the improvement of their economically usage.

Recent researches have focused on the ultrasound combined with other disinfection methods, such as chlorination, ultraviolet radiation (UV), ozone (O_3), electrochemical (EC) disinfection, photocatalytic (PC) applications, and hydrogen peroxide (H_2O_2) to improve its inactivation efficiency for large scale water disinfection plants. Ayyildiz et al. [33] were used US (150 W/L–300 W/L) as a pre-treatment for chlorine dioxide disinfection to solve the high disinfectant dose requirement, and they choosed the US instead of UV or O_3 because suspended particles were listed as important disadvantages of these systems. Loraine et al. [34] compared the disinfection performance of hydrodynamic cavitation (HC) near low frequency US and ultasonic cavitation on Gram (–) *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas syringae*, and *P. aeruginosa*, and Gram (+) *B. subtilis*, they reported that disinfection efficiency of HC was changed from 2-log to 6-log depending bacteria, and the HC was 10–100 times more effective than ultasonic cavitation [34]. Gogate et al. [35] developed a hybrid reactor combined HC, O_3, US, and EC methods to use in the wide range of water and wastewater treatment applications. They explained their theoretical claims for expected synergism of hybrid application with hydroxyl radical concentration produced in these methods. Similar to this study, Naddeo et al. [36] was used the hybrid water disinfection of US and UV as an alternative method to chlorination and to improve the efficiency covering disadvantages of UV for low transmittance wastewater. They indicated that total coliform and *E. coli* with 10^4 CFU/L concentration were disinfected reducing fouling formation on UV lamps. In addition, Gogate [37] reviewed the US disinfection, the operating parameters of US, reactor design of US, and hybrid applications of US detailly. Mason et al. [38] performed synergistic application of chlorine (1 ppm) and US (38 kHz, 0.6 W/cm^2 power density) in a continuous system, and they stated that ultrasound could require to be combined with another disinfection treatment, such as chlorine, O_3, or UV for the effective bacterial inactivation using single-pass treatments almost certainly [38]. In parallel with this work, Phull et al. [39] examined the combined chlorine (70 mg/L) and US (20, 38, and 40 kHZ applying 5 W/cm^2 power density) disinfection to reduce the amount of chlorine required for effective inactivation of bacteria resulting lower by-product risks [39]. Besides, the photo-Fenton (1 ppm Fe^{+2}, 10 ppm H_2O_2, and 800–1,200 W/m^2 light intensity) improved with US (40 kHz, 20 W) disinfection was performed by Giannakis et al. [40] to enhance the *E. coli* inactivation of photo-Fenton for incessant effect due to direct physical damage of US, chemical inactivation effect of US, direct DNA damage of UV, and hydroxyl radical accelerated with photo-Fenton mechanisms [40]. Joyce et al. [41] observed the synergistic effect of US (40 kHz, 0.05 W/cm^3), and EC (100 and 150 mA) disinfection providing the *K. pneumonia* suspensions mixing, the mechanical effect of US on bacterial cells, and the electrode surface cleaning with ultrasound.

In these entire hybrid studies summarized above, US and Ag ion were combined with other disinfection methods to overcome their listed disadvantages. This paper has focused on the water disinfection using US and Ag ion to promote ultrasonic inactivation efficiency and decrease Ag amount required for effective bacterial removal by using their environmentally and economically hybrid applications for the first time in the literature. In this study, *K. pneumoniae* was disinfected using three different ultrasonic reactors, which were operated with three frequencies at continuous flow conditions and three Ag ions concentrations. This study has stated that hybrid application US and Ag ion was a successful, effective, healthy, and advantageous water disinfection method.

2. Methods

Three different ultrasonic reactors were designed for determining the disinfection efficiencies of ultrasound (US) and silver ion (Ag^+) along with the hybrid application (US + Ag^+) of these two systems. The experimental details were explained in microbiological studies, ultrasonic disinfection studies, silver ion disinfection, and hybrid disinfection sections.

2.1. Microbiological studies

Gram (–) *K. pneumoniae* bacteria were used to perform disinfection experiments with ultrasound (US), silver ion (Ag^+), and hybrid application (US + Ag^+) for water treatment. The working solution with the total volume of 100 mL was prepared with 1 × 10^4 CFU/mL initial bacteria concentration of *K. pneumoniae* for disinfection application of ultrasound, silver ion, and their hybrid systems. Disinfection studies were performed in Hereous KSP-18 class II sterile cabinet in order to prevent external microbiological contaminations (Thermo Scientific, Massachusetts, USA) in experiment conditions and all equipment have been sterilized within the Nuve 40 autoclave (Nüve, Ankara, Turkey) for a period of 20 min at 121°C.

Firstly, the samples were collected from three disinfection processes at specified retention time were diluted and afterwards these samples were inoculated plate count agar media (PCA, Merck, Darmstadt, Germany), which is the general media to count of bacteria cultivated. After inoculation of the samples, the plates were incubated at 37°C in Innova-42 shaker series incubator with 18–24 h [42,43]. Lastly incubation, the colonies growing in the surface of plate were counted to calculate average bacterial concentration of each sample. The inactivation yields of three systems were determined with Eq. (1) accounting to percent and Eq. (2) accounting to logarithmic term stated by Karel [43].

$$E(\%) = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

where E (%) is the bacterial inactivation efficiency (%), C_0 is the average concentration of *K. pneumonia* at time 0 (CFU/mL), C_t is the average concentration of *K. pneumonia* at time t (CFU/mL):

$$E(\log) = \log\left(\frac{C_0}{C_t}\right) \quad (2)$$

where E (log) is the bacterial inactivation efficiency (%), C_0 is the average concentration of *K. pneumonia* at time 0 (CFU/mL), C_t is the average concentration of *K. pneumonia* at time t (CFU/mL).

Three independent experiments were performed and the average results were given to illustrate the disinfection efficiency of US, Ag^+, and US + Ag^+ treatments. The analysis of variance (ANOVA) using one-way test in groups and using two-way test between groups were examined to statistical significance of microbiological studies.

2.2. Disinfection studies with ultrasound

Ultrasonic disinfection studies were performed in three different continuous flow reactors with 50 mL volume. The first reactor, USR22, the second reactor, USR36, and the third reactor, USR833, have ultrasonic frequencies with 22, 36, and 833 kHz, respectively, given in Fig. 1. The RF power amplifier (electronics – innovation with 1140LA model number) and the single channel arbitrary function generator (Tektronix AFG3021 B), (Tektronix, Berkshie, UK) were used for ultrasonic disinfection studies.

The temperature of working solution was set at room temperature during ultrasonic disinfection process with cooling water passed through the outer wall of the reactor using a water bath (PolyScience, Illinois, USA) in order to prevent temperature effect resulting from ultrasonic cavitation on bacterial disinfection. In this study, *K. pneumoniae* was disinfected using three different ultrasonic reactors, which were operated with three frequencies (22, 36, and 833 kHz) at continuous flow condition (5, 15, and 25 mL/min) applying different power (36, 51, 67, and 95 W). The optimization of system parameters, which are applied power, flow rate, and hydraulic retention time with bacterial inactivation ratio were determined in a continuous flow ultrasonic reactor in USR 22. USR36 and USR 833 were operated with optimum conditions determined in USR22. During disinfection period the samples were collected from ultrasonic process, and then the disinfection efficiencies of USR22, USR36, and USR833 were determined using bacterial inactivation ratio calculated by Eqs. (1) and (2).

2.3. Disinfection studies with silver ion

The disinfection studies performed with using silver ion whose concentrations were limited with regard to water and health regulations of silver ion, the effect of silver ion concentration on disinfection process was researched with 0.1, 0.01, and 0.005 mM Ag^+ concentration. Their stock solutions with 0.5, 1 mM, and 10 mM Ag^+ concentration were freshly prepared in PP volumetric flasks which are autoclavable at 121°C by using $AgNO_3$, which is chosen as a source of Ag^+ due to its maximum solubility in water among other silver salts. The determined concentrations of Ag^+ were adjusted by transferring 1 mL volumes from suitable fresh stock solutions to the working solution. Doing so, the effects of three concentration of Ag^+ on disinfection of *K. pneumoniae* were researched during disinfection period.

In the disinfection process with silver ion, the working solution in the PP beaker was mixed with a magnetic stirrer with a view to having the bacteria contact with silver ions. During 10 min disinfection period the samples were collected from silver disinfection process with time intervals determined as 1 min. The disinfection efficiencies of 0.1, 0.01, and 0.005 mM Ag^+ concentrations were determined by using bacterial inactivation ratio calculated by Eqs. (1) and (2).

All apparatuses used in the silver disinfection process have been cleaned with %10 nitric acid and rinsed in distil water to remove silver ion absorbed on the surfaces of these apparatuses [44]. After disinfection, the water was centrifuged at 5,000 rpm for 5 min and the disinfected bacteria were separated from the water. The supernatant was analyzed with inductively coupled plasma mass spectrometry (ICP-MS; Agilent 8800 ICP-QQQ, Hanover, Germany) to determine silver concentration in water. Also, the subbacterial suspension was analyzed with scanning electron microscopy (SEM, Hitachi TM3030Plus Benchtop, Tokyo, Japan) and energy dispersive X-ray spectrometry ((EDX, Oxford Instruments Swift ED3000, Oxon, UK). In the regrowth studies, the samples were taken from disinfected water with 0.1, 0.01, and 0.005 mM Ag^+ concentration to ensure that these concentrations were adequate for a bactericidal effect. The samples taken from disinfected water

a b c

Fig. 1. Ultrasonic reactors USR22 (a), USR36 (b), and USR833 (c).

with 0.1, 0.01, and 0.005 mM Ag^+ concentration during 144 h were inoculated PCA media and incubated at 37°C in Innova-42 shaker series incubator with 18–24 h.

2.4. Disinfection studies with hybrid system

The hybrid system studies were carried out three ultrasonic reactors, which have 22, 36, and 833 kHz frequencies with addition of 0.1, 0.01, and 0.005 mM Ag^+. After disinfection period, the samples have collected from hybrid disinfection process according to flow rates and hydraulic retention time. The disinfection efficiencies of 0.1, 0.01, and 0.005 mM Ag^+ concentrations at three different ultrasonic reactors have been determined with using bacterial inactivation ratio calculated by Eqs. (1) and (2).

3. Results and discussion

In this study, *K. pneumoniae* disinfection efficiencies were determined using US, Ag^+, and their hybrid applications. The data of these experiments were summarized and disgusted in results of disinfection studies with ultrasound, silver ion, and hybrid system.

3.1. Results of disinfection studies with ultrasound

The result of ultrasonic disinfection studies performed with 100 mL working solution, which has 1 × 10^4 CFU/mL average *K. pneumoniae* concentration in USR22 with different power application in the continuous flow conditions is presented in Fig. 2.

The result of ultrasonic disinfection studies performed in USR22, USR36, and USR833 operated with 95 W powers in the continuous flow conditions is presented in Fig. 3 to indicate ultrasonic frequency effect on disinfection.

The result of ultrasonic disinfection studies performed in USR22 with 95 W powers in the three different flow rates (*A*) and hydraulic retention time (*B*) is presented in Fig. 4 to determine the contact time effect in continuous flow condition. In this study, the bacterial inactivation efficiency occurred in ultrasonic disinfection studies was affected the applied power (R^2 = 0.9952), the ultrasonic frequency (R^2 = 0.8666) linearly. In parallel with previous studies in literature, the maximum bacterial inactivation efficiency was obtained in USR22 because its frequency is between high power ultrasound frequencies.

In ultrasonic disinfection, the bacterial inactivation can occur in three ways. The first way is the mechanical collapse of the single bacterial cell by the local high pressure and temperature effect created by ultrasound. The second system is the mechanical dispersion of the bacteria colony and the creation of single bacteria cell for effective inactivation. The last way is the sonochemical disinfection with hydrogen peroxide released by ultrasonic hydrolysis of water. Karel [42] reported that 28 kHz ultrasonic frequency was more effective than respectively 45 and 100 kHz for the disinfection of *E. coli* applying same power density (100 w/100 mL). Joyce et al. [31] investigated that ultrasonic disinfection performed 20 and 38 kHz frequencies

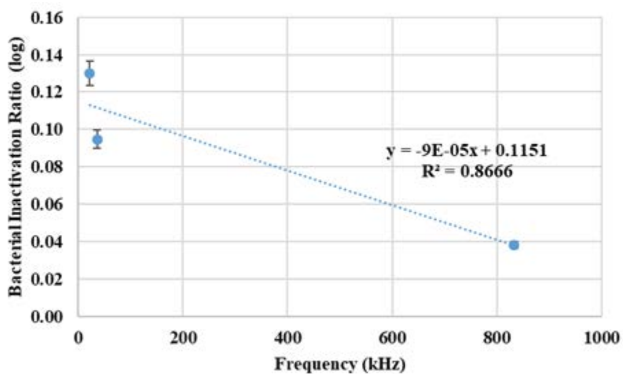

Fig. 3. Bacterial inactivation ratio occurred in three ultrasonic reactors with different ultrasonic frequencies (USR22, USR36, and USR833 operated with 95 W power and 5 mL/min flow rate in the continuous flow conditions with 1 × 10^4 CFU/mL initial *Klebsiella pneumoniae* concentration) (f = 3.7 and f_{critic} = 5.14 using one-way ANOVA test in groups).

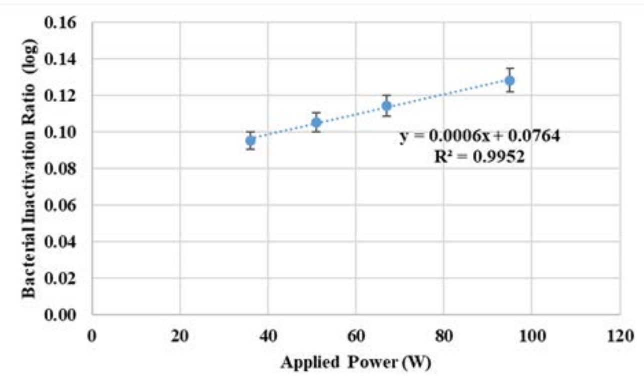

Fig. 2. Ultrasonic disinfection efficiency of three ultrasonic reactors with different power application (USR22 operated with 22 kHz frequency and 5 mL/min flow rate in the continuous flow conditions with 1 × 10^4 CFU/mL initial *Klebsiella pneumoniae* concentration) (f = 1.23 and f_{critic} = 4.07 using one-way ANOVA test in groups).

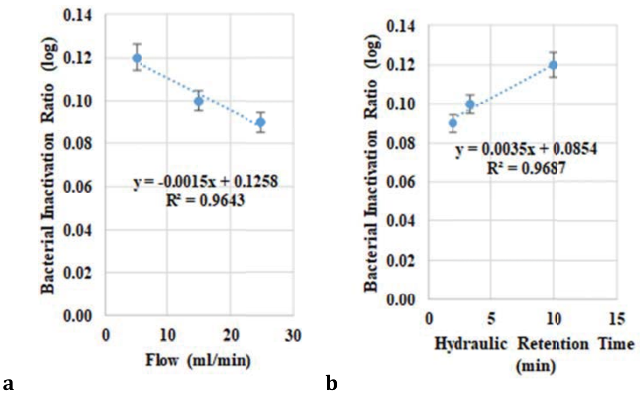

Fig. 4. Ultrasonic bacterial inactivation ratio obtained with three flow rates (a) and three hydraulic retention time (b) (USR22 operated with 95 W power and with 1 × 10^4 CFU/mL initial *Klebsiella pneumoniae* concentration) (f = 1.46 and f_{critic} = 5.14 using one-way ANOVA test in groups).

increased inactivation ratio of *Bacillus* species rather than 512–850 kHz frequencies [31]. Gómez-López et al. [45] used high power ultrasound described as using low ultrasonic frequencies for *E. coli* disinfection and indicated that high power density provides high bacterial inactivation with simultaneous temperature effect. Joyce et al. [46] investigated that 20 and 40 kHz ultrasonic frequencies are more effective for *E. coli* and *K. pneumoniae* than 580 kHz frequency because high power ultrasound predominantly creates cell membrane disruption. Mechanical disinfection rather than sonochemical disinfection, as seen in the study performed by Mason et al. [47] was more effective in the same power application. In the ultrasonic disinfection process, bacterial inactivation was raised with increasing power. It is the major known disadvantage of ultrasonic disinfection stated in many studies that high energy consumption is required to achieve high inactivation rate [48]. These summarized studies demonstrated that low-frequency ultrasound applications, were more effective in microbial disinfection because of mechanical depletion of bacteria and separation of bacteria colonies.

The effectiveness of the third sonochemical disinfection mechanism was examined in the literature. Phull et al. [39] investigated the disinfection of *E. coli* (10^3 CFU/mL) using four different ultrasonic frequency (20, 38, 40, and 800 kHz) and different intensity (5, 12, 15, and 20 W/cm^2) combined with chlorination. They reported that high-frequency US (800 kHz) was more effective than low-frequency US (20, 38, and 40 kHz) thanks to sonochemical inactivation mechanisms. Their results showed that ultrasound could be used efficiently for water disinfection with several advantages. When the US was used with chlorine, it significantly reduced the number of bacteria in water samples, and use of ultrasound decreased the amount of chlorine required for disinfection [39]. Koda et al. [48] compared the power US (20 kHz) and high-frequency US (500 kHz) to disinfect the *E. coli* (10^8 CFU/mL) and *Streptococcus mutans* (10^6 CFU/mL), and they indicated that the high-frequency US (500 kHz) was more effective than the power US (20 kHz). Inez and Thompson [49] used the power US (20 kHz) referring most effective frequency for *E. coli* (3–6 × 10^6 CFU/mL) disinfection using 0.46 W/cm^3 power density, they attained 4-log *E. coli* removal.

In addition to the studies summarized above, the important review papers comparing of ultrasonic disinfection studies performed under different conditions are also available in literature. For instance, Matafonova and Batoev [50] detailly reviewed the effectiveness of the low-frequency US disinfection and high-frequency US disinfection in their study in which sonophotochemical inactivation of pathogenic microorganisms was suggested as promising method to raising the inactivation efficiency and reduce energy consumption of US.

The inconsistencies in the literature indicated the requirement of more detailed analyses about the effect of ultrasonic frequency. In this study, the effect of ultrasonic frequency was examined with three different US frequencies at the same power application, operating parameters, bacteria, and experimental conditions. Obviously, more than three data might be more useful to establish linearity between bacterial inactivation and ultrasonic frequency; however this article provided preliminary findings obtained from ultrasonic reactors designed at three different frequencies operated in the same conditions. Thus, these limited data demonstrated the possible trend of the ultrasonic frequency on microbial inactivation.

The bacterial inactivation efficiency was affected the retention time (R^2 = 0.9687) and flowrates (R^2 = 0.9643) linearly. In the reactors operated under continuous flow condition, the residence time is determined by the flowrate and reactor volume. In the disinfection studies carried out by changing the flowrate, it was determined that the bacterial inactivation rate decreased linearly as the flowrate increased. Therefore, it was observed that the inactivation rate increased linearly as the residence time determined by using flow rate and reactor volume. As expected, the rise in residence time increased the exposure time of the bacteria to the ultrasound in the reactor and improved the inactivation rate. In addition, as the residence time increased, the colony of bacteria was mechanically dispersed and formed more single bacterial cells, resulting in a higher inactivation rate.

3.2. Results of disinfection studies with silver ion

The disinfection studies performed with using silver ion researched with 0.1, 0.01, and 0.005 mM Ag^+ concentration. The result of silver ion disinfection studies is indicated in Fig. 5 and the results of the regrowth studies are shown in Fig. 6.

As expected, increasing the silver ion concentration was accelerated the bacterial inactivation in Fig. 4. When a 0.005 mM silver ion concentration was used for disinfection, a 1.3-log disinfection efficiency was reached with a 2 min disinfection period. When used silver ion concentration was 0.01 mM, a 2.4-log disinfection efficiency was achieved with a 2 min disinfection period. When silver ion concentration was increased to a 0.1 mM, the inactivation ratio of *K. pneumoniae* was obtained as 4-log with a 2 min disinfection period. Application of 10 min disinfection period, it was clearly stated that the 0.005 mM silver ion concentration was adequate for comprehensive inactivation.

The results of silver ion disinfection studies, changing silver ion concentration in water affects directly disinfection efficiency and disinfection period depends on

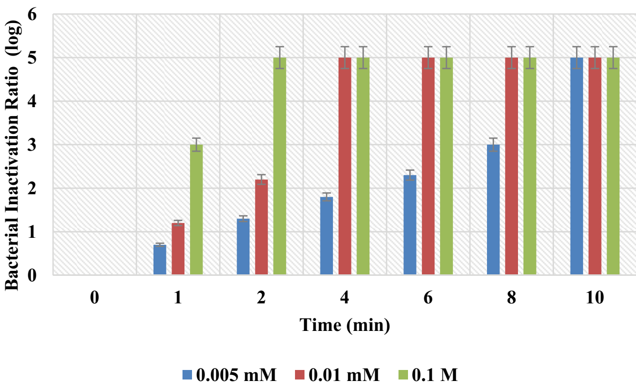

Fig. 5. Silver ion concentration effects on *Klebsiella pneumoniae* disinfection (f = 2.20 and f_{critic} = 6.94 using one-way ANOVA test in groups).

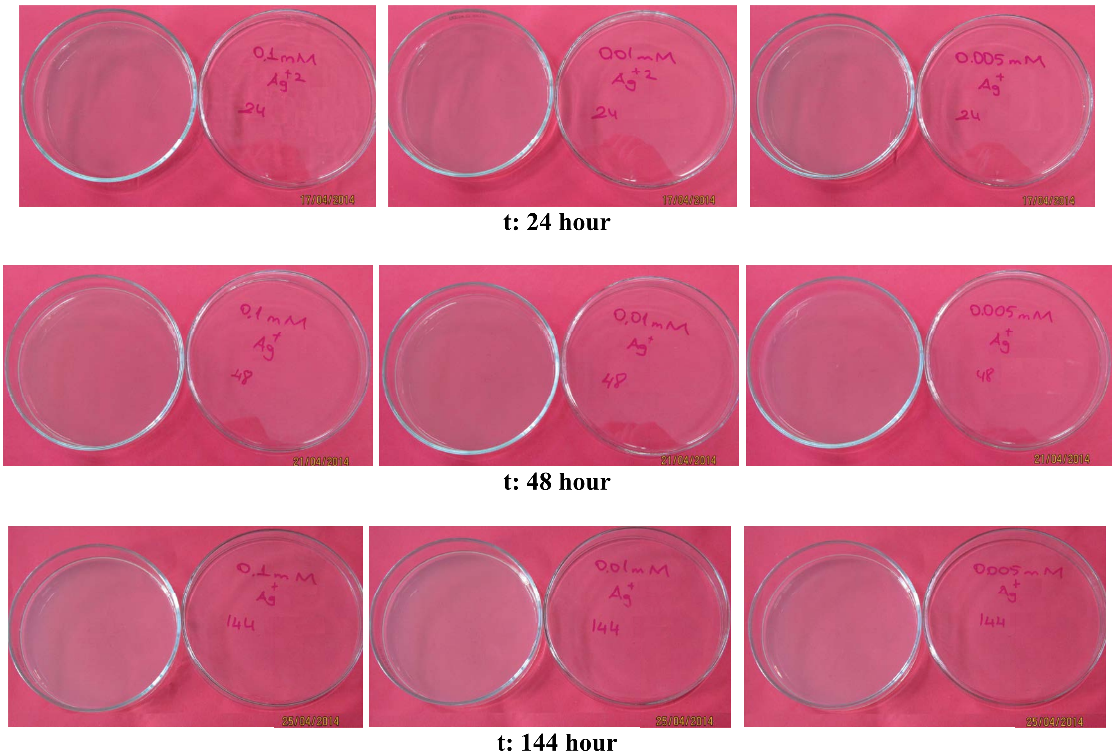

Fig. 6. Silver ion concentration effects on *Klebsiella pneumoniae* disinfection and regrowth control.

the microorganisms and the size of silver. Yoon et al. [51] examined the sensitivity of *E. coli* and *B. subtilis* to silver and copper NPs. The results of the experimental studies were determined that *B. subtilis* showed more sensitivity than *E. coli* against both silver and copper nanoparticles comparing of the nanoparticle susceptibility constant (Z, mL/μg), and higher Z-value was indicated that bacteria were more sensitive to NPs. They reported that the Z-value was equal to 0.0734 mL/μg for *B. subtilis* with 100 nm copper NPs while the Z-value was 0.0236 mL/μg for *E. coli* with 40 nm silver NPs [51].

When the silver ion concentration decreased from 0.1 to 0.005 mM Ag^+ concentration, the disinfection time was reached to 10 from 2 min for the 4-log bacterial inactivation ratio. The silver ion concentration required for microbiological inactivation can be increased to accomplish rapid decontamination, but it must be considered the silver ion concentration in water allowed by water and health regulation to escape its environmental health risks. No growth was observed on the petri dish even after 144 h in the regrowth experiments in Fig. 6. The samples taken from disinfected water with 0.1, 0.01, and 0.005 mM Ag^+ concentration during 144 h showed that these silver ion concentrations were adequate for a bactericidal effect. When silver ions are sufficiently absorbed by the bacteria, the bacteria become inactivated as a result of cellular destruction. This shows that the concentrations of silver ions used in the disinfection experiments provide the MIC required for bacterial inactivation. It was stated that the MIC varied depending on the type of organism to be inhibited, the type of silver used, and the particle size of the silver when the disinfection studies carried out with silver, in the previous studies [52].

3.3. Disinfection studies with the hybrid system

As a result of hybrid application ultrasound and silver ion with the purpose of determining three ultrasonic reactors, which have 22, 36, and 833 kHz frequencies with addition of 0.1, 0.01, and 0.005 mM Ag^+ were stated in this part. The hybrid disinfection study performed in USR222 with adding 0.1, 0.01, and 0.005 mM Ag^+ concentrations during 5 min disinfection period is shown in Fig. 7.

When hybrid application including 0.01 mM Ag^+ concentration, the same disinfection efficiency of single silver treatment with 0.1 mM Ag^+ concentration after 3 min disinfection period. Furthermore, in the USR22 + 0.005 mM Ag^+ hybrid disinfection provided 4 log disinfection efficiency of a single silver ion with 0.1 mM Ag^+ concentration after 5 min disinfection period. This means that hybrid application can be successfully decreased 10–20 times whole silver quantity released to the water-related with disinfection period and silver ion concentrations.

The hybrid application results of other ultrasonic reactors (USR36 and USR 833) with the same conditions with USR22 after 5 min disinfection are shown in Fig. 8 and two-way ANOVA test is given in Table 1. The SEM and EDX results of disinfected *K. pneumoniae* within the USR22 + 0.005 mM Ag^+ hybrid application are shown in Figs. 9 and 10.

The mechanical disruption effects of low-frequency US enhanced the disinfection efficiency by improving the chemical inactivation effect of silver synergistically. Like USR22, 4 log *K. pneumoniae* inactivation efficiency was achieved with ultrasound and silver ion hybrid application in the USR36 and USR833 successfully. The efficiency

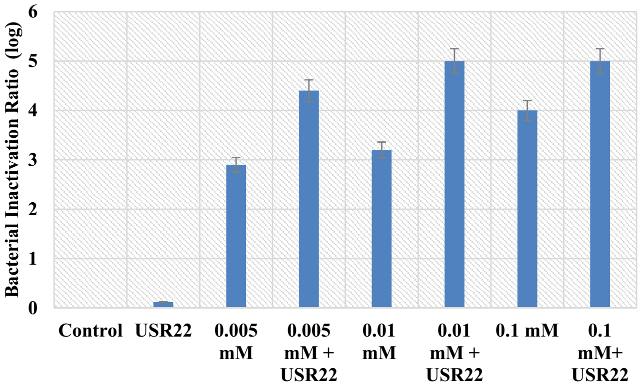

Fig. 7. Hybrid disinfection study in USR22 (f = 2.42 and f_{critic} = 4.10 using one-way ANOVA test between groups).

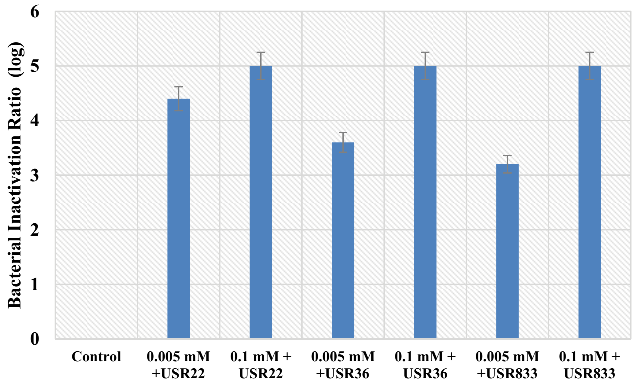

Fig. 8. Hybrid application silver ion in USR22, USR36, and USR833 after 5 min disinfection.

Table 1
ANOVA results of US + Ag^+ hybrid application

ANOVA: two way						
Summary			Number of groups	Total	Average	Varian
Trial 1			7	101,080	14,440	1,456,901,325
Trial 2			7	96,026	13,718	1,314,853,446
Trial 3			7	106,134	15,162	1,606,233,711
Control			3	303,000	101,000	25,502,500
0.005 mM + USR22			3	15	5	0.0625
0.1 mM + USR22			3	0	0	0
0.005 mM + USR36			3	75	25	1.5625
0.1 mM + USR36			3	0	0	0
0.005 mM + USR833			3	150	50	6.25
0.1 mM + USR833			3	0	0	0
Sources of variation	SS	df	MS	F	P-value	f_{critic}
In groups	7,297,976	2	3,648,988	1.00184904	0.395942	3.885294
Between groups	26,224,223,850	6	4,370,703,975	1,200	5.92E-16	2.99612
Total	26,275,228,866	20				

of the hybrid application performed in USR22 was equal nearly USR36 and higher than USR833. Thus, high-power US effect on bacterial disinfection efficiency was stated in hybrid application ultrasound and silver ions with synergistically. These results point out that synergistic effect of US and Ag^+ hybrid application was reduced silver ion concentration to attain same disinfection yield in water for the reason that US increase speed of inactivation of bacteria exposed to Ag^+ via silver ion mass transfer from cell membrane [53]. In addition, the ultrasound separates the bacterial colonies, allowing the silver ion to reach all the bacteria in the colony. The mechanical disinfection effect of ultrasound combined with the chemical disinfection mechanism of the silver ion enabled all bacteria to be inactivated in such a way that no re-growth was observed.

In Table 1, the results of ANOVA using two-way test, p-value was determined 0.396 within groups and p-value was calculated 5.9210^{-16} between groups. According to these results, there is no significant difference within groups because p-value was greater than 0.01. Also, US + Ag^+ hybrid

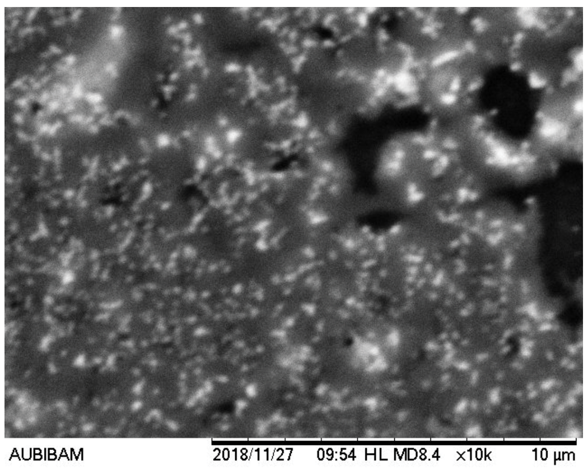

Fig. 9. SEM image of *Klebsiella pneumoniae* disinfected in USR22 with silver ion after 5 min disinfection (20 kVA, 8.4 working distance, and 10,000× magnification).

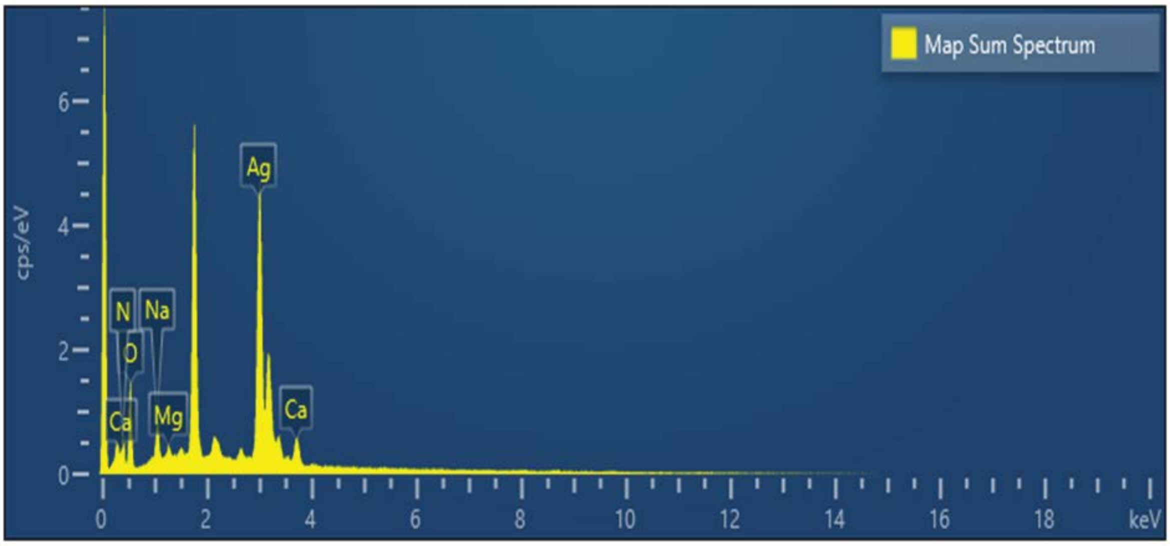

Fig. 10. EDX results of *Klebsiella pneumoniae* disinfected in USR22 with silver ion after 5 min disinfection (20 kVA, 8.4 working distance, and 10,000× magnification).

disinfection applications provided consistent and repeatable results.

In the SEM image, the bacterial cells were seen in pale gray areas as with organic structure and bright coloured regions showed inorganic silver ions. Bacterial cells did not demonstrate clearly due to the simultaneous visualization of the organic and inorganic structures and the need for 20 kVA for EDX result. As a result of the ICP-MS analysis with the 0–10 μg/L calibration range, DL: 0.045 μg/L detection limit, and R^2 = 0.9997 linearity in the He collision mode, it was determined that the Ag content of supernatant was lower than the detection limit. When the SEM image in Fig. 9, EDX results in Fig. 10 were examined in addition to the ICP-MS results, it was determined that the almost silver ions applied to water were absorbed by the bacteria. It was stated that the bacteria adsorbed the silver ion onto their cell membrane, bacterial inactivation occurs with disruption of cell. When silver ion concentration reaches to killing density onto their cell membrane, water was disinfected efficiently.

4. Conclusion

In this paper, the bacterial disinfection efficiency was obtained using US + Ag^+. The highest *K. pneumoniae* inactivation ratio was determined in USR22 with 22 kHz frequencies, which indicates high power ultrasound. Disinfection of *K. pneumoniae* with ultrasound can be performed in a short contact time effectively. The US + Ag^+ provides faster and more efficient bacterial inactivation than single applications of silver. US reduced the silver amount used to inactivate same bacterial concentration. As a result, this work points out that the hybrid application ultrasound is a successful water disinfection method using silver ion with lower concentration with the purpose of developing effective, healthy, and advantageous water treatment methods. The single treatment process both silver and ultrasound indicated that synergic effect was not only formed ultrasound. Increasing disinfection efficiency with higher silver concentration was expected result but hybrid disinfection studies were performed to determine lowest silver concentration requiring bacterial inactivation with ultrasound.

For future work, disinfection efficiency of US and silver can be determined for other microorganism in separate, consequent, and hybrid application of these two methods. The effect of dissolved gas in water (like nitrogen and oxygen) and effect of anions and cations can be presented in water body must be clearly stated on US + Ag^+ or US. The effect of bacterial concentration in water can be investigated on US, Ag^+, or US + Ag^+. The toxicity of US + Ag^+ and Ag^+ treatments can be compared with a quick bacterial test. The disinfection efficiencies of US + Ag^+ and Ag^+ treatments might be investigated with large-scale studies of microbial inactivation while eliminating the disadvantages of these systems to use water treatment successfully.

Acknowledgments

In 2015, US reactors used in this study were patented Turkish Patent Institute with 2011/03602 patent number in the category of SECTION E – FIXED CONSTRUCTIONS.

Part of this study was presented in the conference of the European Society of Sonochemistry held in "Besancon," France.

References

[1] N. Gordon, J. Economy, Nanometallic particles for oligodynamic microbial disinfection, Nanotechnol. Appl. Clean Water, 3 (2009) 3–15, doi: 10.1016/B978-0-8155-1578-4.50010-X.
[2] B. Horst, F.H.H. Brill, Antimicrobial efficacy of modern wound dressings: oligodynamic bactericidal versus hydrophobic adsorption effect, Wound Med., 5 (2014) 16–20.
[3] S. Rtimi, M. Pascu, R. Sanjines, C. Pulgarin, M. Ben-Simon, A. Houas, J.C. Lavanchy, J. Kiwi, ZrNO–Ag co-sputtered surfaces leading to *E. coli* inactivation under actinic light:

evidence for the oligodynamic effect, Appl. Catal., B, 138 (2013) 113–121.
[4] T. Schmidt-Braeklin, A. Streitbuerger, G. Gosheger, F. Boettner, M. Nottrott, H. Ahrens, B. Moellenbeck, Silver-coated megaprostheses: review of the literature, Eur. J. Orthop. Surg. Traumatol., 27 (2017) 483–489.
[5] J.L. Clement, P.S Jarrett, Antibacterial silver, Met.-Based Drugs, 1 (1994) 467–482.
[6] B. Van Aken, L.S. Lin, Effect of the disinfection agent's chlorine, UV irradiation, silver ions, and TiO_2 nanoparticles/near-UV on DNA molecules, Water Sci. Technol., 64 (2011) 1226–1232.
[7] F.X. Abad, R.M. Pinto, J.M. Diez, A. Bosch, Disinfection of human enteric viruses in water by copper and silver in combination with low-levels of chlorine, Appl. Environ. Microbiol., 60 (1994) 2377–2383.
[8] Q. Bao, D. Zhang, P. Qi, Synthesis and characterization of silver nanoparticle and graphene oxide nanosheet composites as a bactericidal agent for water disinfection, J. Colloid Interface Sci., 360 (2011) 463–470.
[9] K. Zodrow, L. Brunet, S. Mahendra, D. Li, A. Zhang, Q. Li, P.J. Alvarez, Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal, Water Res., 43 (2009) 715–723.
[10] W. Zhang, X.Z. Zhang, Adsorption of MS2 on oxide nanoparticles affects chlorine disinfection and solar inactivation, Water Res., 69 (2015) 59–67.
[11] H.Y. Zhang, V. Oyanedel-Craver, Comparison of the bacterial removal performance of silver nanoparticles and a polymer based quaternary amine functiaonalized silsesquioxane coated point-of-use ceramic water filters, J. Hazard. Mater., 260 (2013) 272–277.
[12] H.Y. Zhang, V. Oyanedel-Craver, Evaluation of the disinfectant performance of silver nanoparticles in different water chemistry conditions, J. Environ. Eng. ASCE, 138 (2012) 58–66.
[13] M.F. Brugnera, M. Miyata, C.Q.F. Leite, M.V.B. Zanoni, Silver ion release from electrodes of nanotubes of TiO_2 impregnated with Ag nanoparticles applied in photoelectrocatalytic disinfection, J. Photochem. Photobiol., A, 278 (2014) 1–8.
[14] I. De la Rosa-Gomez, M.T. Olguin, D. Alcantara, Antibacterial behavior of silver-modified clinoptilolite-heulandite rich tuff on coliform microorganisms from wastewater in a column system, J. Environ. Manage., 88 (2008) 853–863.
[15] L.P. Lukhele, R.W. Krause, Z.P. Nhlabatsi, B.B. Mamba, M.N. Momba, Copper and silver impregnated carbon nanotubes incorporated into cyclodextrin polyurethanes for the removal of bacterial and organic pollutants in water, Desal. Water Treat., 27 (2011) 299–307.
[16] O. Akhavan, E. Ghaderi, Self-accumulated Ag nanoparticles on mesoporous TiO_2 thin film with high bactericidal activities, Surf. Coat. Technol., 204 (2010) 3676–3683.
[17] R. Bandyopadhyaya, M.V. Sivaiah, P.A. Shankar, Silver-embedded granular activated carbon as an antibacterial medium for water purification, J. Chem. Technol. Biotechnol., 83 (2008) 1177–1180.
[18] X.X. Zhao, T. Toyooka, Y. Ibuki, Synergistic bactericidal effect by combined exposure to Ag nanoparticles and UVA, Sci. Total Environ., 458 (2013) 54–62.
[19] S. Agnihotri, S. Mukherji, S. Mukherji, Immobilized silver nanoparticles enhance contact killing and show highest efficacy: elucidation of the mechanism of bactericidal action of silver, Nanoscale, 5 (2013) 7328–7340.
[20] R.K. Sharma, S. Kumar, R.W. Ramteke, P.K. Ray, Disinfection of drinking-water by filtration through silver impregnated alumina, J. Environ. Sci. Health., Part A Environ. Sci. Eng. Toxic Hazard. Subst. Control, 25 (1990) 479–486.
[21] M. Azimzadehirani, M. Elahifard, S. Haghighi, M. Gholami, Highly efficient hydroxyapatite/TiO_2 composites covered by silver halides as *E. coli* disinfectant under visible light and dark media, Photochem. Photobiol. Sci., 12 (2013) 1787–1794.
[22] N.T.T. Hoang, N.V. Suc, T.V. Nguyen, Bactericidal activities and synergistic effects of Ag-TiO_2 and Ag-TiO_2-SiO_2 nanomaterials under UV-C and dark conditions, Int. J. Nanotechnol., 12 (2015) 367–379.
[23] S. Khan, I.A. Qazi, I. Hashmi, M.A. Awan, N.U.S.S. Zaidi, Synthesis of silver-doped titanium TiO_2 powder-coated surfaces and its ability to inactivate *Pseudomonas aeruginosa* and *Bacillus subtilis*, J. Nanomater., 2013 (2013), 1–9 doi: 10.1155/2013/531010.
[24] R. Khaydarov, R. Khaydarov, B. Yuldashev, Experience of using energy-effective water disinfection devices, Water Supply Emergency Situations, 1 (2007) 127–132.
[25] X. Qu, J. Brame, Q. Li, P.J. Alvarez, Nanotechnology for a safe and sustainable water supply: enabling integrated water treatment and reuse, Acc. Chem. Res., 46 (2013) 834–843.
[26] R. Manjumeena, D. Duraibabu, J. Sudha, P.T. Kalaichelvan, Biogenic nanosilver incorporated reverse osmosis membrane for antibacterial and antifungal activities against selected pathogenic strains: an enhanced eco-friendly water disinfection approach, J. Environ. Sci. Health., Part A Toxic Hazard. Subst. Environ. Eng., 49 (2014) 1125–1133.
[27] M.L. Pedro-Botet, I. Sanchez, M. Sabria, N. Sopena, L. Mateu, M. García-Núñez, C.R. Joly, Impact of copper and silver ionization on fungal colonization of the water supply in health care centers: implications for immunocompromised patients, Clin. Infect. Dis., 45 (2007) 84–86.
[28] A. Alonso, X. Munoz-Berbel, N. Vigués, J. Macanás, M. Munoz, J. Mas, D.N. Muraviev, Characterization of fibrous polymer silver/cobalt nanocomposite with enhanced bactericide activity, Langmuir, 28 (2012) 783–790.
[29] H. Basri, A.F. Ismail, M. Aziz, Polyethersulfone (PES)-silver composite UF membrane: effect of silver loading and PVP molecular weight on membrane morphology and antibacterial activity, Desalination, 273 (2011) 72–80.
[30] H. Barani, M. Montazer, N. Samadi, T. Toliyat, Nano silver entrapped in phospholipids membrane: synthesis, characteristics and antibacterial kinetics, Mol. Membr. Biol., 28 (2011) 206–215.
[31] E. Joyce, S.S. Phull, J.P. Lorimer, T.J. Mason, The development and evaluation of ultrasound for the treatment of bacterial suspensions. a study of frequency, power and sonication time on cultured *Bacillus* species, Ultrason. Sonochem., 10 (2003) 315–318.
[32] P. Declerck, L. Vanysacker, A. Hulsmans, N. Lambert, S. Liers, F. Ollevier, Evaluation of power ultrasound for disinfection of both *Legionella pneumophila* and its environmental host *Acanthamoeba castellanii*, Water Res., 44 (2010) 703–710.
[33] O. Ayyildiz, S. Sanik, B. Ileri, Effect of ultrasonic pretreatment on chlorine dioxide disinfection efficiency, Ultrason. Sonochem., 18 (2011) 683–688.
[34] G. Loraine, G. Chahine, C.T. Hsiao, J.K. Choi, P. Aley, Disinfection of gram-negative and gram-positive bacteria using DYNAJETS (R) hydrodynamic cavitating jets, Ultrason. Sonochem., 19 (2012) 710–717.
[35] P.R. Gogate, S. Mededovic-Thagard, D. McGuire, G. Chapas, J. Blackmon, R. Cathey, Hybrid reactor based on combined cavitation and ozonation: from concept to practical reality, Ultrason. Sonochem., 21 (2014) 590–598.
[36] V. Naddeo, M. Landi, V. Belgiorno, R.M.A. Napoli, Wastewater disinfection by combination of ultrasound and ultraviolet irradiation, J. Hazard. Mater., 168 (2009) 925–929.
[37] P.R. Gogate, Application of cavitational reactors for water disinfection: current status and path forward, J. Environ. Manage., 85 (2007) 801–815.
[38] T.J. Mason, S.S. Phull, J.P. Lorimer, Potential uses of ultrasound in the biological decontamination of water, Ultrason. Sonochem., 10 (2003) 319–323.
[39] S.S. Phull, A.P. Newman, J.P. Lorimer, B. Pollet, T.J. Mason, The development and evaluation of ultrasound in the biocidal treatment of water, Ultrason. Sonochem., 4 (1997) 157–164.
[40] S. Giannakis, S. Papoutsakis, E. Darakas, A. Escalas-Cañellas, C. Pétrier, C. Pulgarin, Ultrasound enhancement of near-neutral photo-Fenton for effective *E. coli* inactivation in wastewater, Ultrason. Sonochem., 22 (2015) 515–526.
[41] E. Joyce, T.J. Mason, S.S. Phull, J.P. Lorimer, The development and evaluation of electrolysis in conjunction with power ultrasound for the disinfection of bacterial suspensions, Ultrason. Sonochem., 10 (2003) 231–234.

[42] F.B. Karel, Disinfection of *Klebsiella pneumoniae* using ultrasonic systems, J. Environ. Biol., 37 (2016) 10–13.
[43] F. Karel, *Klebsiella pneumoniae* disinfection with ultrasound and hydrogen peroxides, Appl. Ecol. Environ. Res., 17 (2019) 4159–4169.
[44] M.A. Butkus, M.P. Labare, J.A. Starke, K. Moon, M. Talbot, Use of aqueous silver to enhance inactivation of coliphage MS-2 by UV disinfection, Appl. Environ. Microbiol., 70 (2004) 2848–2853.
[45] V.M. Gómez-López, M.I. Gil, A. Allende, J. Blancke, L. Schouteten, M.V. Selma, Disinfection capacity of high-power ultrasound against *E. coli* O157:H7 in process water of the fresh-cut industry, Food Bioprocess Technol., 7 (2014) 3390–3397.
[46] E. Joyce, A. Al-Hashimi, T.J. Mason, Assessing the effect of different ultrasonic frequencies on bacterial viability using flow cytometry, J. Appl. Microbiol., 110 (2011) 862–870.
[47] T.J. Mason, A.J. Cobley, J.E. Graves, D. Morgan, New evidence for the inverse dependence of mechanical and chemical effects on the frequency of ultrasound, Ultrason. Sonochem., 18 (2011) 226–230.
[48] S. Koda, T. Kimura, T. Kondo, H.A. Mitome, A standard method to calibrate sonochemical efficiency of an individual reaction system, Ultrason. Sonochem., 10 (2003) 149–156.
[49] H. Inez, J.E. Thompson, Inactivation of *Escherichia coli* by sonication at discrete ultrasonic frequencies, Water Res., 34 (2000) 3888–3893.
[50] G. Matafonova, V. Batoev, Review on low-and high-frequency sonolytic, sonophotolytic and sonophotochemical processes for inactivating pathogenic microorganisms in aqueous media, Water Res., 166 (2019) 1–11, doi: 10.1016/j.watres.2019.115085.
[51] K.Y. Yoon, J.H. Byeon, J.H. Park, J. Hwang, Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles, Sci. Total Environ., 373 (2007) 572–575.
[52] S. Chernousova, M. Epple, Silver as antibacterial agent: ion, nanoparticle, and metal, Angew. Chem. Int. Ed., 52 (2013) 1636–1653.
[53] D.K. Tiwari, J. Behari, Biocidal nature of combined treatment of Ag-nanoparticle and ultrasonic irradiation in *Escherichia coli* DH5, Adv. Biol. Res., 3 (2009) 89–95.