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Escherichia coli Disinfection with Ag^+ in the Recycled Flow Ultrasonic Reactor

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Escherichia coli;
Ultrasound;
Silver ion;
Recycled flow

Abstract

After the recent pandemic process, disinfection/sterilization methods used for the control of infectious diseases have gained even more importance. The alternative disinfection studies like ultrasound (US) have been developing due to the various shortcomings of microbial inactivation processes such as chlorine application, ozone and ultraviolet radiation, which are widely applied for the prevention of epidemic diseases caused by pathogenic microorganisms. Within the scope of this study, disinfection treatments were carried out in recycled flow ultrasonic reactors operated at 22kHz, 36kHz and 833kHz ultrasonic frequencies using *Escherichia coli* bacteria indicated fecal contamination in the water sources. In this article, the combined usage of silver ions (0.1mM, 0.01 mM and 0.005 mM Ag^+) and US (22 kHz, 36 kHz and 833 kHz ultrasonic frequencies) were mainly investigated using initial *Escherichia coli* bacteria concentration of $1x10^4$ CFU/mL. As a result of the study, *Escherichia coli* was inactivated obtaining 4-log reduction with 22 kHz ultrasonic frequency with the minimum Ag^+ concentrations. To conclude, the disinfection was effectively achieved with US and Ag^+ methods, and higher *Escherichia coli* inactivation rate were obtained in US-Ag^+ dual processes with faster decontamination time and lower Ag^+ concentration due to their synergistic effects.

Geri Döngü Akışlı Ultrasonik Reaktörde Ag^+ ile *Escherichia coli* Dezenfeksiyonu

Anahtar kelimeler
Dezenfeksiyon;
Escherichia coli;
Ultrases;
Gümüş iyonu;
Geri döngülü akış

Öz

Son zamanlarda yaşanan pandemi sürecinin ardından bulaşıcı hastalıkların kontrolü için kullanılan dezenfeksiyon/sterilizasyon yöntemleri daha da önem kazanmıştır. Patojenik mikroorganizmaların neden olduğu salgın hastalıkların önlenmesinde sıklıkla kullanılan klor uygulamaları, ozon ve ultraviyole gibi mikrobiyal inaktivasyon süreçlerinin sahip olduğu olumsuz etkiler nedeniyle ultrases (US) gibi yeni dezenfeksiyon yöntemleri geliştirilmektedir. Bu çalışma kapsamında 22kHz, 36kHz ve 833kHz ultrasonik frekanslarda çalıştırılan geri döngülü akışlı ultrasonik reaktörlerde su kaynaklarında fekal kontaminasyon varlığını işaret eden *Escherichia coli* bakterisi kullanılarak dezenfeksiyon işlemleri gerçekleştirilmiştir. Bu çalışmada esas olarak, gümüş iyonlarının (Ag^+) (0.1 mM, 0.01 mM ve 0.005 mM Ag+) US ile (22 kHz, 36 kHz ve 833 kHz ultrasonik frekanslar) hibrit kullanımı $1x10^4$ CFU/mL başlangıç *Escherichia coli* bakteri konsantrasyonu kullanılarak araştırılmıştır. Çalışma sonucunda *Escherichia coli* minimum Ag^+ konsantrasyonunda ve 22 kHz ultrasonik frekansta 4-log giderim verimi elde edilerek inaktive edilmiştir. Sonuç olarak, US ve Ag^+ yöntemleriyle dezenfeksiyon etkili bir şekilde sağlanmış ve US-Ag^+ ikili proseslerinde, sinerjik etkileri nedeniyle daha hızlı dekontaminasyon süresi ve daha düşük Ag^+ konsantrasyonu ile daha fazla *Escherichia coli* inaktivasyon oranı elde edilmiştir.

1. Introduction

Microorganisms can be defined with the simplest approach as microscopic organisms ranging in size from 1 to 100 µm. It is known that the presence of disease-causing (pathogenic) bacteria and viruses in waters attitudes an important health risk. Microbial diseases such as typhoid, cholera and viral hepatitis cause thousands of people to get sick or die every year. The disinfection studies applied to prevent infectious diseases have been increasing recently. For instance, the number of studies on inactivation such as chlorination, ozonation, and UV irradiation, which are widely applied to prevent epidemic diseases caused by pathogenic microorganisms, and alternative hybrid and sequential inactivation technologies have gained importance in water supply (Spellman 1999). The major parameters for the determination of disinfection alternatives; effectiveness, cost, feasibility, bench-scale treatment prerequisite and lateral effects. The effectiveness of the disinfection method is to ensure that the selected microorganisms are inhibited at the target and reliable level (Singer 1994). The sterilization chemicals used in the water are chlorine compounds like gas form (Cl_2) and salt forms ([$Ca(OCl)_2$] and (NaOCl), and dioxide forms (ClO_2). Another disinfectant used is ozone due to its strong disinfection effect even at low concentrations (Al-Hamzaha *et al*. 2019). Along with these, hydrogen peroxide and ammonium quaternary salts are also used for this purpose (Bláhová *et al*. 2021). The most important variable in the chemical sterilization process listed above is the contact time according to Chick Law, which is frequently used as the simplest model for microbial inactivation (Manoli *et al*. 2019). The effectiveness of disinfectants is also affected by the types of microorganisms. For example, growing bacteria can be easily killed, while the bacterial spores are very resistant to chemical disinfectants. Therefore, other disinfection methods are preferred for these types of resistant microorganisms. The emerging ultrasonic sterilization applications are promising for these resistant microbes (Li *et al*. 2019).

Ultrasound is formed as a result of vibrations created by molecules in an environment during their movements. The ultrasound waves are longitudinal waves with frequencies above the audible order (>16-18 kHz) obtained by applying an alternating electric field to a crystallin surfaces like quartz (Mason 1990). In this way, it is possible to obtain ultrasonic frequencies as high as $6x10^8$ Hz equal to 600 mHz. Ultrasonic application can be divided into low- frequency and high-frequency ultrasound. High-frequency ultrasound waves in the 2- 10 mHz range are used in the field of health due to their low energies. Low-frequency ultrasound, which is described in the 20-1000 kHz wave range, attracts wide attention and finds application in various fields such as chemical, physical and environmental applications (Leighton 2007). When ultrasound waves hit the surface of a liquid medium, the molecules in the liquid creates micro-sized bubbles via the vapor of the liquid or the air in the environment by making vibration, rotation and pushing movements. These bubbles are called as cavitations, and these cavitations can tend to fade as temporary cavitations or stable cavitations. The damping of temporary cavitations is called collapse and releases the energy trapped inside the cavitations. In ultrasonic disinfection systems; there is a need the formation of temporary cavitations instead of stable cavitations since the energy created as a result of collapses is used for microbial cell damage (Mason *et al*. 2003). If the external pressure is high, cavitations are difficult, but collapses occur easily and are more severe. These events are local and instantaneous, and the period of cavitations is 10^{-8} seconds. This creates high local temperature and pressure, which can rupture bacteria cells and destroy their enzymes. The rapidly immersing bubbles also produce high shear forces and liquid jets in the water, which similarly have adequate sonic-intensity to physically detriment their wall or membrane (Leighton 1998). Also, chemical disinfection occurs with the formation of radicals (H^+ and OH^-) during cavitation in water environments. These radicals disrupt the microbial cell and deteriorate the cell wall. The final hydrogen peroxide, which is a strong disinfectant, was produced sono-chemically (Ziembowicz *et al*. 2018). Sonication provides a powerful disinfection, but it is required high ultrasonic intensity to achieve 100% inactivation using ultrasound alone. This can make the technique costly to use in large-scale microbial decontamination (Khaire *et al*. 2022). Joyce *et al*. (2003) studied about the *Bacillus subtilis* inactivation with sonication and investigated the outcome of sonic frequency and practical power on the US microbial inactivation obtaing the the highest bacterial inactivation ratio with ultrasonic frequencies of 20 and 38 kHz. However, the use of hybrid systems with ultrasound in decontamination in addition to other techniques such as chlorination, UV and metal ions, can also reduce the cost of ultrasound lowering disadvantages of listed conventional disinfection methods. Sonication including hybrid and consecutive applications were more preferred in the previous study (Gogate 2007). Phull *et al*. (1997) determined the effect of sonication on microorganism removal from water. They reported that sonication and chlorination had a more efficient bacterial inactivation, and using their hybrid application reduced the required amount of chlorate and achieved the same inactivation efficiency. The researches indicate that enhancing more microbial utilization need more intensity (Joyce *et al*. 2003) and so, the investigations on ultrasonic dual and trial processes reduce the cost of energy and chemical requirements (Chon *et al*. 2012). Recent researches focus on the sonication combined with other treatments methods such as chlorine compound, UV, O_3, H_2O_2, in order to use their synergistic features (Ibarluzea *et al*. 1998, Naddeo *et al*. 2009, Jyoti and Pandit 2004, . Giannakis *et al*. 2015).

Metal ions can be used for microbial inactivation with high cidal effectiveness. As alternative disinfection technology to conventional water disinfection methods, Cu, Ag, Ti, and Co are the most preferred to disinfect the pathogens (Akhavan, and Ghaderi 2010). These metals are effective for the numerous pathogens even if they are applied with lower than 1-ppm concentration due to their oligodynamic characters (Basri *et al*. 2011). Silver ion (Ag^+) inactivates numerous species varying from coliforms to Gram (+) and Gram (-) bacteria, human enteric viruses and yeasts (Van Aken, and L. S. Lin 2011, Agnihotri *et al*. 2013, Qu *et al*. 2013). Nawaz *et al*. (2012) carried out disinfection with 0.01-0.1 mg/L Ag^+ concentrations for *Escherichia coli* and *P. aeroginosa*, which are frequently encountered in rainwater. They achieved a 95-99% reduction after 10-14 hours for *P. aeroginosa*, and a 95-99% reduction after 8-24 hours for *E. coli*. They observed re-growth at the end of 168 hours at silver concentrations lower than 0.04 mg/L for both bacterial strains. For this reason, they emphasized that Ag^+ concentration should be applied above this value for bactericidal effect. Furthermore, antimicrobial materials produced with Ag nanoparticles needing advance technology have attracted consideration of most researchers because they have minor Ag^+ amount like 1-2%. Shihang *et al* (2013) conducted a study on the disinfection of drinking water with Ag nanoparticle alginate alloy beads, and they achieved 5 log removal by performing *Escherichia coli* disinfection in 1-minute hydraulic holding time by producing three different alginate beads at different silver concentrations. Brugnera *et al* (2014) conducted a study on the application of photocatalytic disinfection with electrodes formed from TiO_2 nanotubes doped with 0.05 M $AgNO_3$. In their article, they achieved a 99.6% reduction after 3 minutes for *M. smgnatis.* Consequently, these goods preserve their long time- microbial reduction effects continually and economically with decreasing ecological risks (Gross *et al*. 2012). Ag^+ which has strong bacterial inactivation efficiency at short disinfection period is trained with other disinfection methods, particularly TiO_2 or ZnO (Hoang *et al*. 2015) to decrease Ag^+ refraining health risks of its usages.

When the studies on disinfection with US and Ag^+ were examined in the literature, it was clearly seen that hybrid inactivation processes were preferred in order to eliminate the disadvantages listed above for both disinfection methods. However, there was unique article in which these two methods were used together (Ozmen and Koparal 2021). The necessity of determining the synergistic effect of these methods has occurred on different species as indicator microorganisms can be found in the environment. For these reasons, in this article, it was aimed to examine the disinfection of *Escherichia coli* bacteria with single and hybrid methods with US and Ag^+ in the recycled flow ultrasonic reactor.

2. Material ve Methods

The experimental details were explained in this section.

2.1 Bacteriological Experiments

In this article, which the inactivation productivities of ultrasound (US), silver ion (Ag+) and synergistic usage (US+Ag+) of these techniques were investigated for water treatment, and Gram (-) *Escherichia coli* bacteria were used to perform disinfection experiments. The total volume of 100 mL was used as working solution with $1x10^4$ CFU/mL of *Escherichia coli* for obtaining disinfection efficiency. The microbial studies were completed in sterile cabinet (Thermo Hereous KSP-18 Class II) and all equipment have been decontaminated with in the autoclave (Nuve 40). The treatment setup was illustrated in the Figure 1.

Figure 1. Treatment setup

The aliquots were diluted, and then, they inoculated plate count agar media (PCA, Merck) to determine the amount of bacteria cell. After injection of the samples, the plates were incubated at 37 °C in incubator (Innova-42 Shaker Series) with 18-24 hour. After the cultivation, the bacterial colonies rising in the outward of plate were calculated as the average bacteria concentration of each three disinfection treatments.

2.2 Bacterial Disinfection Experiments

US inactivation experiments were achieved in reactors with 22 kHz, 36 kHz, and 833 sonic frequencies respectively. The RF power amplifier (Electronics -Innovation, 1140LA) was used with 95 W power applied with sole station random function generator (Tektronix AFG3021 B).

Disinfection process was carried in the recycled flow ultrasonic reactor operated with 75 ml/min flowrate. The temperatures of reactors were established at room temperature during the experiment with external cooler system to avoid heat effect of ultrasound. The treatment scheme was given in the Figure 2.

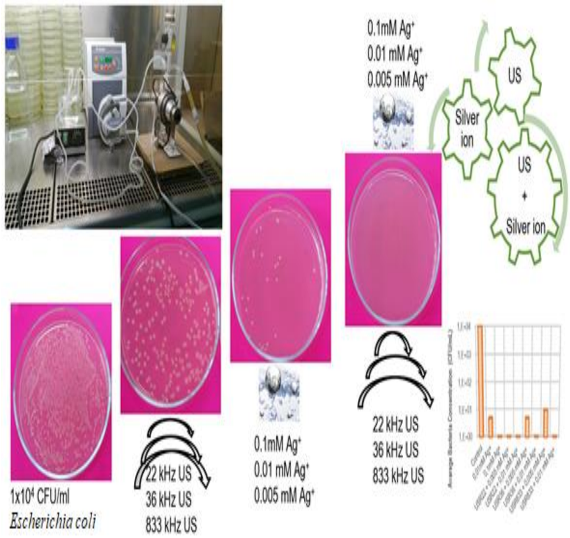

Figure 2. Figure 2 Treatment scheme for *Escherichia coli* disinfection with (US) and (Ag^+) and ($US+Ag^+$) hybrid application

The inactivation experiment achieved with Ag^+ whose concentrations were restricted with respect to water and health guidelines of Ag^+, the effect of Ag^+ concentration on bacterial inactivation was examined with 0.1mM, 0.01 mM and 0.005 mM Ag^+ concentration. Their primary standard solutions with 0.5 mM, 1 mM and 10 mM Ag^+ concentration was newly set in polypropylene volumetric flasks. The desired concentrations of Ag^+ were attuned transferring 1 mL volumes from primary standard solutions to the working solution. The synergistic experiments were carried out with three ultrasonic reactors with addition of 0.1mM, 0.01 mM and 0.005 mM Ag^+. Throughout the experiments, the aliquots were collected from the system with desired time intervals.

3. Results and Discussions

The outcome of US inactivation in USR22, USR36 and USR833 in the recycled flow ultrasonic reactor was shown in Figure 3.

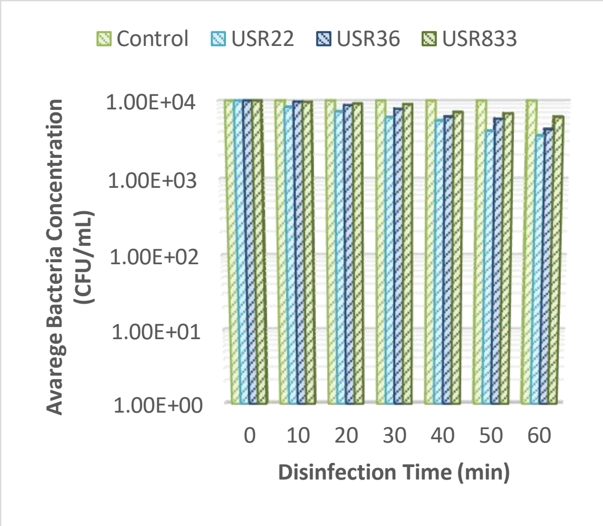

Figure 3. The result of US disinfection according to ultasonic frequency

In this work, the maximum bacterial destruction of *Escherichia coli* was obtained with 22 kHz ultasonic frequency. Many of the previous studies correlated these findings. Scherba *et al*. (1991) investigated the impacts of ultrasound at 24 kHz frequency and different ultrasonic intensity(W/cm^2) in an aqueous solution on *Escherichia coli* inactivation. They reported that as the exposure time increased, the decrease in the bacterial population increased, but increasing the power did not affect the bacterial utilization. Limaye and Coakley (1998) examined the disinfection of *Escherichia coli* and *Saccharomyces cerevisiae* at a rate of 95.5% in 4.5 and 11.5 minutes using 1 or 3 MHz ultrasonic frequency, respectively. In another work, İnce and Belen (2001) presented that *Escherichia coli* was inactivated with 20kHz ultrasonication, and inactivation ratio of *Escherichia coli* was increased with mixing ZnO particles. Furthermore, Antoniadis *et al*. (2007) stated that *Escherichia coli* bacteria applied 24 kHz ultrasonic frequency and 450 W power, resulting in a 3log reduction in 30 minutes of ultrasonic contact time for domestic wastewater. In this article, they stated that sonication was a powerful disinfection method, but high ultrasonic intensity is required to achieve 100% removal. They emphasized that this would be expensive for large-scale disinfection applications. However, they argued that low frequency ultrasound would be appropriate for bacteria resistant to disinfection methods including UV, ozone, electrolysis, thermal disinfection, chlorination and biocides [36]. In similar article, Declerck *et al*. (2010) found that for *Legonella pneumophila* and *Acenthomobea castellani,* were inactivated with 1.3 -1.5 log reduction respectively at the end of 30 min disinfection at 36 kHz ultrasonic frequency and applying 0.064-0.191 kW power per liter. In parallel with previous studies, *Escherichia coli* was more successfully inactivated by low frequency ultrasound due to its mechanical inactivation mechanism rather than sonochemical disinfection occurred at high frequency ultrasound (>500 kHz). It was found that low frequency US (22 and 36 kHz) was more effective for *Escherichia coli* inactivation due to powerful mechanical both unique bacterial cell and bacterial cluster decomposition effect. It apparently examined that the disinfection effect of US was limited (approximately 1-log) even if the mechanical disinfection (22 kHz and 36 kHz) was more effective rather than sonochemical disinfection (833 kHz) in this article.

The disinfection experiments were carried with 0.1mM, 0.01 mM and 0.005 mM Ag^+ concentration. The findings of these experiments were given in Figure 4.

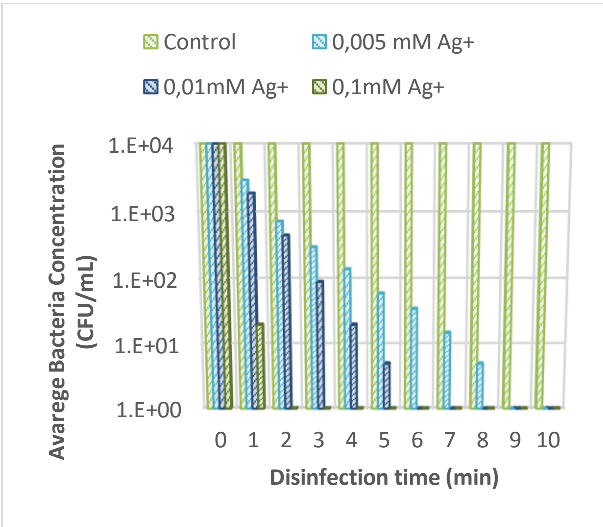

Figure 4. The effects of Ag^+ concentration on Escherichia coli

Escherichia coli was inactivated with 4-log reduction efficiency with 0.1 mM Ag^+ after 2 min. The results of Ag^+ disinfection studies, changing Ag^+ concentration raises the inactivation rate and reduce the inactivation time. Alonso *et al*. (2012)

examined the of Ag^+ and cobalt (Co) nanoparticles against *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus* with an initial concentration 10^5 CFU/mL. Barani *et al*. (2011) stated that the minimum inhibition concentration was 25 µL and minimum bactericidal concentration was 40 µL for nano-sized silver material. In similar article, Sarvar *et al*. (2021) investigated the susceptibility of *Escherichia coli* and *Bacillus subtilis* to nano-sized Ag and Cu particles. They indicated that *Bacillus subtilis* was more sensitive to both Ag and Cu nanoparticles than *Escherichia coli*. It was emphasized that the relationship between nanoparticle size and susceptibility constant should be investigated in further studies because the diameters of nanoparticles impacts their inactivation mechanisms. The reaction of *Bacillus subtilis* with 100 nm Cu nanoparticles showed the 0.0734 mL/µg sensitivity, while *Escherichia coli* showed the 0.0236 mL/µg sensitivity. The previous finding in the literature correlated these results. When decreasing metal ions concentration, the same bacterial inactivation ratio was attained with higher disinfection period. When Ag^+ concentration reaches to killing density in water and bacteria adsorb Ag^+ onto their cell membrane, the inactivation happens with disruption of the bacteria cell. To accomplish influential bacterial inactivation in short disinfection period, Ag^+ amount should be enlarged according to water and health guideline for Ag^+ concentration of water to circumvent its environmental health effect.

The result of hybrid application US and Ag^+ were stated in the Figure 5 to examine the effect of ultrasonic frequency (22 kHz) with Ag^+ (addition of 0.1mM, 0.01 mM and 0.005 mM). According to Figure 5, the similar bacterial utilization was attained in the of Ag^+ application with 0.1 mM concentration and dual US and Ag^+ process after 3 min disinfection. Also, in the USR22 + 0.005 mM Ag^+ dual inactivation created 4-log reduction with 0.1 mM Ag^+ after 5 min sonication. The dual treatment was effectively reduced 10 to 20 times total metal ion amount dropped to the water during inactivation.

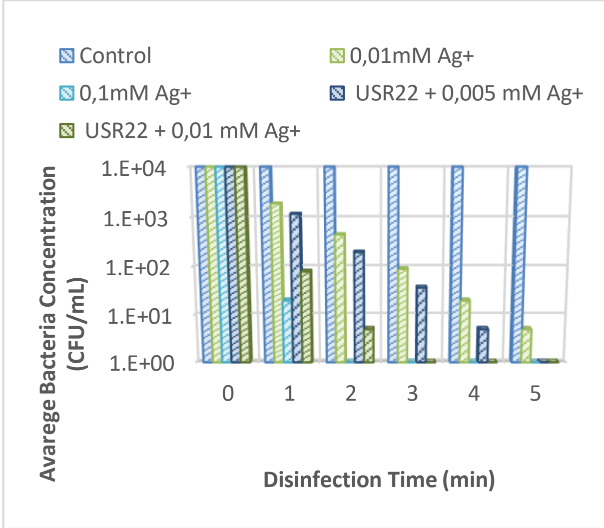

Figure 5. The result of synergistic US and Ag^+ disinfection

In the work conducted by Dadjour *et al* (2006), *Legonella pneumophila* was removed in 30 minutes by applying 36 kHz and 300 W power in a 5.8 L ultrasonic bath with 1 g/mL TiO_2. With the similar aim, Agnihotri *et al*. (2013) immobilized the nano-sized material containing 35 % silver to improve the inactivation providing several reuses. The dual and trial application findings of other ultrasonic frequencies (36 kHz and 833 kHz) with same settings with 22 kHz after 5 min disinfection were illustrated in Figure 6.

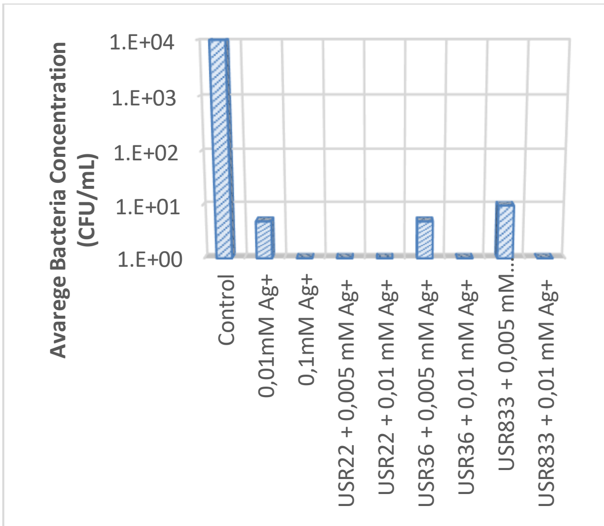

Figure 6. The effect of sonic frequencies on the hybrid application US and Ag^+

4-log inactivation efficiency was obtained for USR22, USR36 and USR833 with 0.01 mM Ag^+ concentration while USR36 and USR833 were not completely remove the bacteria with 0.005 mM Ag^+. The efficiency of dual USR22+Ag^+ treatment was similar with USR36+Ag^+ and higher than USR833+Ag^+. In a supportive manner, Hoang *et al*. (2015) investigated the gel–derived Ag–TiO_2–SiO_2 nanomaterials disinfected the water treatment under both dark and UV-C treatments with dual impacts to obtain acceptable disinfection [31]. Consequently, high power sonication impact on inactivation ratio was attained in dual usage sonication and metal ions. These findings stated that using US like UV-C and Ag^+ dual treatment was concentrated Ag^+ concentration to achieve same bacterial inactivation efficiency in water because synergistic effects of US and UV-C accelerates to contact of Ag^+ with bacteria and Ag^+ mass transfer from cell membrane.

4. Conclusion

This presented work was concluded that *Escherichia coli* inactivation ratio was attained using dual treatment of sonication and Ag^+. In the studies carried out in the ultrasonic reactor, the best results were obtained at 22 kHz frequency. The Ag^+ ion addition provided higher *Escherichia coli* disinfection in a quicker disinfection period than studies performed with US alone. In addition, the essential quantity of Ag^+ for *Escherichia coli* inactivation has been condensed by 50% by the combined use of US and Ag^+.

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5. Referances

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