Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.27835

242 (2021) 89–93
December

Inactivation of *Escherichia coli* in water by using ultrasonic disinfection processes

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Received 22 February 2021; Accepted 23 September 2021

ABSTRACT

The current conventional disinfection for municipal water relies primarily on chlorine disinfection alone, in spite of its high running cost and its production of disinfection by-products. Ultrasonic disinfection is a physical technology widely accepted and continuously appraised as an alternative to conventional techniques for water disinfection. The efficiency of using the ultrasonic disinfection technique was evaluated and compared to the conventional chlorination technique using HOCl. Hybrid treatment was also investigated via the combination of both types of treatment. A laboratory-scale unit was constructed to carry out disinfection experiments using synthetic water (feed solution) prepared with a known concentration of *Escherichia coli*. The results showed that all the tested treatment methods are capable, to some degree, of inactivating *E. coli* in water. However, the best treatment option was achieved when coupling ultrasonic and HOCl for disinfection. This kind of treatment significantly reduces the concentration of bacteria higher than using each treatment alone. Thus, the ultrasonic technique is a better option for disinfection enhancement when combined with HOCl, in terms of inactivation of *E. coli* bacteria in municipal water.

Keywords: Chlorination; Disinfection process; *E. coli* inactivation; Ultrasonic applications; Water treatment

1. Introduction

Water is an essential element of human life. However, it can transmit a wide range of diseases to humans by contamination [1]. The World Health Organization (WHO) statistics indicate that at least 2–2.5 million people around the world die of diarrhea diseases caused by water contamination annually [2]. Therefore, drinking water disinfection plays a vital role in preventing and controlling waterborne pathogens [3].

At present, disinfection agents widely used within drinking water treatment plants are chlorine and its related compounds, such as sodium and calcium hypochlorite and chlorine dioxide, with chlorine being by far the most commonly used disinfectant [4]. Whilst these treatment processes can reduce the number of microorganisms in water, they can never eliminate them, so final disinfection is the most important stage of water treatment, as it is the last line of defense against water-borne microbial disease. Although the efficacy of the inactivation of bacteria by chlorination may be increased by increasing the concentration of hypochlorite, this can exacerbate other problems associated with the use of excess chlorine [5]. In addition, some bacteria in the water appear to form bacterial aggregates. Therefore, only bacteria on agglomerate surfaces can be inactivated by the chlorine disinfection process, while the bacteria in the innermost areas remain intact [6]. As a result, alternative disinfection methods are being evaluated, and the advantages of using ultrasonic in water treatment are of considerable interest [7].

Ultrasonic disinfection (USD) is an attractive, environmentally friendly technique that creates no disinfection by-products. It is a chemical-free mechanism that induces cell disruption by causing cavitation in the solution. Bubbles shape and break, causing variations in turbulence and pressure that can rupture the bacteria [6,8]. However,

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using USD treatment on its own is not only difficult to ensure complete disinfection, but also needs to consume a considerable amount of energy for large-scale disinfection treatment. The combined technology of the USD and other technologies in the field of water disinfection must therefore be investigated [5].

In this study, the efficacy of using USD is compared to the conventional chlorination disinfection technique using synthesis water inoculated with a known amount of *Escherichia coli* bacteria. Combining both disinfection processes will also be studied to determine their efficacy in the inactivation of *E. coli* bacteria.

2. Materials and methods

2.1. Bacterial strain and control samples preparation

Bacterial *E. coli* was isolated from drinking water samples. The Vitek 2 compact device was used to identify isolations according to the manufacturer's instructions for the identification of bacteria. The interest in this kind of bacteria is because it is one of the most commonly used fecal contamination indicators in regulations and guidelines dealing with water quality assessment [9].

The inoculum was prepared by using *E. coli* suspensions. The optical density was measured using an optical density meter compared with 0.5 McFarland standards to maintain uniform cell densities. A mean optical density of 0.1 ± 0.02 was achieved at a 600 nm wavelength. Finally, measurements of bacterial removal percentages were calculated according to the following equation:

$$\text{Bacterial removal}(\%) = \left[\frac{(B-A)}{B}\right] \times 100 \tag{1}$$

where A and B are the optical density values for the sample and the control, respectively.

2.2. Synthetic feed solution

The synthetic feed solution was prepared by inoculating 50 mL of *E. coli* in 1 L of sterile distillate water (DW). In addition, the total organic carbon (TOC) was maintained at a permissible standard limit of 2 mg/L [10]. This was achieved by preparing a stock solution of 1,000 mg C/L by adding 2.127 g of potassium hydrogen phthalate (white and acidic salt compound, $C_8H_5KO_4$, and Sigma-Aldrich Chemie GmbH) in a flask and adding enough deionized water to make it a 1 L solution [11]. In addition, the total dissolved solids (TDS) of the water was set at 500 mg/L (the average value of drinking water TDS in Baghdad), by adding 500 mg of sodium chloride (NaCl), extra pure (HiMedia) to the prepared liter. Finally, the pH of the water was adjusted to 6.5–8.5 according to the Iraqi drinking water standard [10]. During the experiments, the water temperature was kept at an average of 22°C ± 0.4°C by using a set temperature and heater operation to create an optimum condition for all experiments.

2.3. Experimental procedure

The inactivation of *E. coli* bacteria was examined after USD treatment alone, HOCl treatment alone, and after combining both disinfection techniques. All experiments were performed in at least duplicate formats.

2.3.1. Ultrasonic experiments design

Experiments were carried out in a 5 L stainless steel tank, equipped with a digital ultrasonic device (model LUC-405, Daihan Labtech Co., Ltd., Korea), at a constant power of 350 W with a frequency of 40 kHz, Fig. 1. Preliminary tests were conducted to obtain the optimal frequency and amplitude of the instrument, considering operating parameters established in previous studies [3,12]. A series of experiments involved the use of high-frequency sonication of *E. coli* and monitoring the effects on its growth. Before sonication, the concentration of *E. coli* in water synthetic was adjusted to as high as $38.25 \times 10^6 \pm 2.6 \times 10^6$ cell/mL. All the experimental components were placed in an autoclave for disinfection before starting each test. Samples were collected from the ultrasonic reactor at various times during the USD treatment (t = 5, 10, 15, 20, 30, 40, 50, 60 min) and immediately analyzed by counting cells using the optical density technique [13]. All the experiments were conducted at a lab temperature of 25°C. A series of experiments were conducted at a power density of 350 W/L (expressed as ultrasonic power/volume for the ultrasound bath) to prevent change in the experimental condition.

2.3.2. Chlorination experiments design

Chlorination experiments using (HOCl) were conducted at a lab temperature of 25°C. The contact time between the

Fig. 1. Photograph of the lab-scale ultrasonic cleaner power sonic 405 (5 L) for water treatment.

chlorine and the feed solution in the feed tank was adjusted to 0, 5, 10, 15, 20, 30, 50, and 60 min. Samples were then withdrawn from the reactor and immediately analyzed for bacteria using the optical density technique. An initial concentration of chlorine of 2 mg/L was maintained by using a commercially available HOCl of 6% concentration. The chlorine concentration was verified using a DPD (diethyl paraphenylenediamine) chlorine testing instrument. This concentration is lower than what is actually used in the Iraqi drinking water treatment plant of 3 mg/L, to reduce the concentration of disinfection by-products [10].

2.3.3. Combination of ultrasonic and HOCl disinfection

This experiment was designed to evaluate the effect of the hybrid treatment process using both ultrasonic at high-frequency and chlorine at a concentration of 2 mg/L, applying the same series of contact times. Bacteria removal was evaluated during experiments that involve physio-chemical disinfection. Experiments using USD treatment alone and HOCl disinfection alone have been considered as control experiments.

2.4. Statistical analysis

An analysis of variance (ANOVA) test was implemented to evaluate the significant differences among the three methods at a *P*-value of 0.05. The statistical analysis was conducted using Microsoft Excel 2010. Quantitative data were expressed as mean ± standard deviation (SD).

3. Results

3.1. Ultrasonic experiments

Synthetic feed water samples were disinfected using ultrasonic treatment to reduce bacteria concentrations at a contact time ranging from 5 to 60 min at 25°C. The results are shown in Fig. 2.

The obtained results show that ultrasonic can be used effectively for water disinfection and the inactivation of *E. coli* bacteria increases with increasing the contact time. After 60 min of contact time, the reduction percentage was 79%.

3.2. Chlorination experiment

The results of treating synthetic water with dosages of 2 mg/L of HOCl at contact times from 5 to 60 min at 25°C are shown in Fig. 3.

The average bacteria concentration was declined following the same trend obtained with ultrasonic treatment. Contact time of 60 min yields an 80% removal percentage.

3.3. Hybrid treatment

Hybrid treatment using ultrasonic with high input power density and chlorine at a concentration of 2 mg/L at the same series of contact times was conducted. The testing results are shown in Fig. 4.

The best result of product water was obtained at contact time 60 min where the main bacterial concentration was decreased to $5.25 \times 10^6 \pm 1.1 \times 10^6$ cell/mL. In general, the hybrid treatment is much more efficient than each treatment alone. The hybrid treatment reduces bacterial concentration by 8%–86% more than the single treatment. In addition, statistical analysis showed significant differences among these treatments at ($p \leq 0.05$).

4. Discussion

The inactivation of bacteria was investigated after using high-frequency ultrasonic, and results suggest that ultrasonic at this frequency are capable of some degree of inactivating bacteria in water, as shown in Fig. 1.

According to a previous study [6], the bacterial reduction in synthetic water samples after the USD was very small in the first 10 min of treatment. However, the reduction began to grow rapidly after 15 min of treatment. It is primarily due to the energy supplied by ultrasonic that has been used to disperse or break down bacterial aggregates. Similar results were obtained from literature where

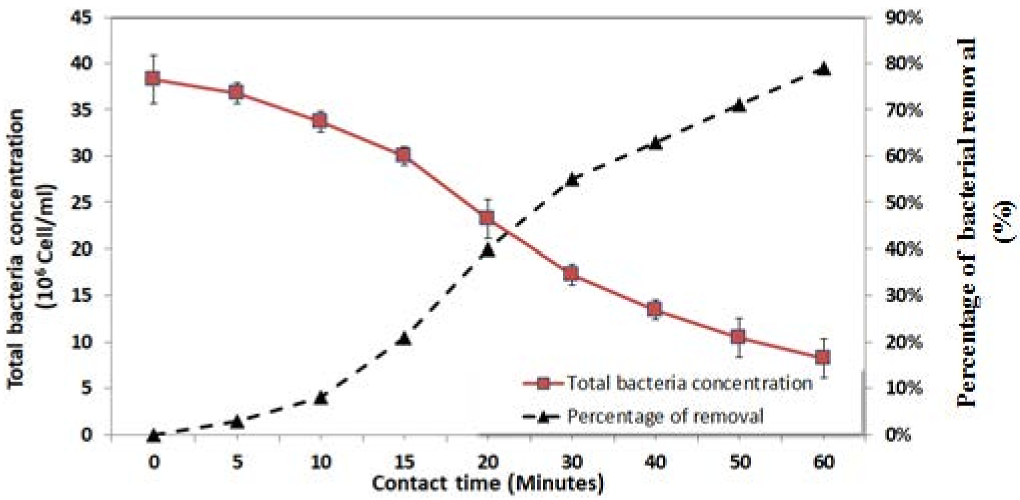

Fig. 2. Results for ultrasonic treatment for feed water to reduce bacterial concentration at several contact time.

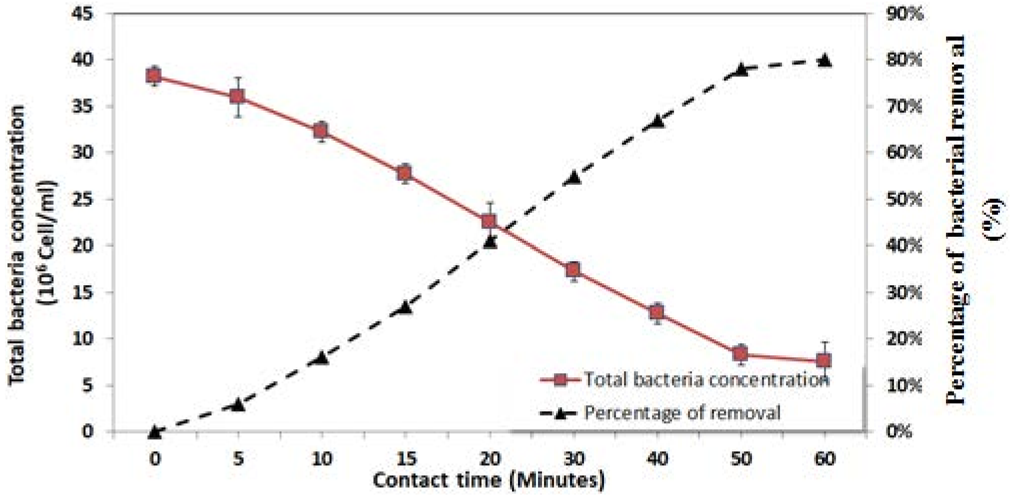

Fig. 3. Results for chlorination treatment for feed water to reduce bacterial concentration at several contact time.

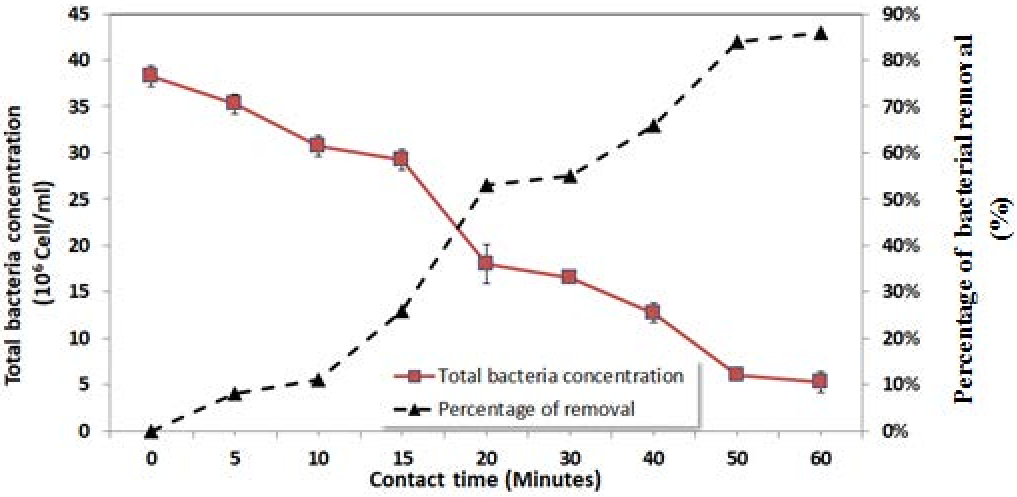

Fig. 4. Results for hybrid treatment for feed water to reduce bacterial concentration at several contact time.

similar experiments were performed to verify the effects of ultrasonic on *E. coli* inactivation, and it was observed that increasing ultrasonic treatment time has a significant effect on bacteria inactivation [9].

In addition, Lambert et al. [14] found that bacteria inactivation was proportional to ultrasonic frequency under their tested conditions, while ultrasonic inactivates pathogens by the physical impact on bacteria depends mainly (high temperature, shear force, and pressure) or the hydroxyl ions and free radicals are produced by oxidizing essential components of bacteria (lipids, proteins, and genetic material) induced during cavitation in the water [15,16]. However, hydroxyl radicals have a very short lifetime and tend to combine to form H_2O_2 as a product that is not considered a carcinogen [17]. The results of this study came in agreement with the effects of using USD in the purification of wastewater reported by Zou and Tang [6], who stated that the USD alone can significantly remove bacteria from wastewater. In addition, an ultrasonic application would significantly reduce the demand for conventional water treatment for this purification and could also prevent the use of chemical agents that could be harmful to the health of ecosystems, such as chlorine [13]. The bacterial inactivation by ultrasound was attributed to heat, mechanical stresses associated with ultrasonically induced cavitation, and uncharacterized synergistic effects [6]. Furthermore, a previous study [18] discovered that the number of *E. coli* cells decreased as the power density of treatments increased due to the dependence of power density on power and volume, which has an effect on bacteria, so the same power density was used in all experiments.

Fig. 3 shows that long contact time allows long interaction opportunities between chlorine and bacteria, and yields a successful disinfection method. The required contact time for the inactivation of bacteria depends on chlorine concentration, the form of pathogens present, the pH, and the water temperature (where the HOCl volatilizes faster from water at elevated temperatures) [19]. In contrast, Ghernaout [20] has claimed that when chlorine, especially HOCl are used in water purification, their favorable effect is in the deactivation of pathogenic and indicator bacteria. However, it produces some harmful disinfection byproducts. For this reason, it is important to use a new method that can give the best results for water purification.

The combination of ultrasonic and HOCl treatment is more effective in reducing bacterial density compared to ultraviolet treatment alone and HOCl alone. However, the additional ultrasonic instruments to conventional treatment will increase the investment cost of the treatment plant. Nevertheless, ultrasonic disinfection is safer, reduces the need for the continuous consumption of disinfection chemicals, and reduces the running cost of the treatment plant.

5. Conclusion

The results suggest that the ultrasonic disinfection technique is capable of inactivating *E. coli* bacteria and yields a bacterial removal of up to 79%. Almost similar results were obtained when using chlorine disinfection by using HOCl. Hybrid treatment obtained from coupling both techniques represents an interesting solution and a better option for disinfection enhancement when combined with HOCl, in terms of inactivation of *E. coli* bacteria in municipal water. Although the addition of ultrasonic instruments increases the investment cost, it can help to reduce the running cost by reducing the consumption of chlorine used for disinfection, and reducing the production of the disinfection by-products. However, further in-depth investigation into the suggested combination is recommended for future research work.

Acknowledgements

We are grateful to everyone who helped us to conduct this study at the Environment and Water Directorate, Ministry of Science and Technology in Baghdad, Iraq. This research did not receive any specific grants from public, commercial or nonprofit organizations.

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