Research Paper

Performance evaluation of combined ultraviolet-ultrasonic technologies in removal of sulfonamide and tetracycline resistant *Escherichia coli* from domestic effluents

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ABSTRACT

Proper treatment of wastewater is key to the achievement of sustainable environmental management. The use of ultraviolet radiation and ultrasound have continued to be considered as some of the best sustainable practices in wastewater purification. However, despite the suitability of the two emerging techniques in sustainably increasing the purification efficiencies of wastewater, their application has not been fully understood, especially in eliminating faecal pathogenic microorganisms. Moreover, their combined potential in the elimination of *Escherichia coli* resistant genes from wastewater has not been adequately explored. This study was designed to evaluate the potential of individual and combined/integrated ultraviolet radiation and ultrasonic technologies in the removal of antibiotic-resistant *E. coli* from domestic effluents. There was a statistical difference in the mean log units of sulfonamide resistant *E. coli* between the different ultraviolet radiation and ultrasonic dosages ($P < 0.05$), showing that ultraviolet radiation technology was more effective in the removal of both sulfonamide and tetracycline resistant *E. coli* from the wastewater. However, the integrated ultraviolet radiation-ultrasonic technique was highly efficient and is recommended in the removal of antibiotic resistant *E. coli* from wastewater. Nonetheless, further studies also need to be performed to further evaluate the disinfection effectiveness on a different bacteria species under continuous operation.

Key words | sulfonamide, tetracycline, ultrasonic, ultraviolet radiation, wastewater

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INTRODUCTION

The critical final process in the management of wastewater and excreta for the protection of human health is disinfection, and pathogens in wastewater can be inactivated or destroyed by either chemical or physical processes (Okoh & Igbinosa 2010). The impact of water-related illness has continued to rise. Indeed, a greater proportion of diarrheal infections is linked to the contamination of drinking water sources by inappropriately treated wastewater (Rop *et al.* 2014). Wastewater is any water that has been degraded in quality by human intervention. Often, wastewater is being treated for re-use as drinking water or for other purposes. Close monitoring and control of the virulence and antimicrobial resistant bacterial populations is of paramount significance in order to better understand their potential public health risk and appropriate control strategies (Kennedy *et al.* 2018). Multi-drug resistance has been found

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to be common among isolates of *E. coli* pathotypes, and the resistance is positively related with virulence (Donde *et al.* 2020). Indeed, most drugs can clear the specific pathotype but cannot completely clear the bacteria from the host due to their resistance. This calls for intervention to ensure that the resistant bacteria are cleared from both their host and source environments. Kordatou *et al.* (2015) showed the potential use of the UV-carbon activated persulfate oxidation process in total inactivation of erythromycin resistant *E. coli* (Liu *et al.* 2017). So far, many waste management institutions and agencies are focusing on pollution control and prevention to the existing drinking water sources. Indeed, proper management of wastewater has remained key to the achievement of sustainable environmental management (Vithanage *et al.* 2016). Studies have focused on the use of sustainable technologies to ensure efficient wastewater treatment (Donde & Bangding 2017; Donde *et al.* 2018; Hui *et al.* 2018). The use of ultraviolet (UV) radiation and ultrasound (US) have continued to be considered as some of the best sustainable practices in wastewater management (Zhang *et al.* 2017).

The UV radiation disinfection technology in wastewater treatment plants involves the instantaneous neutralization of the microorganisms as they pass, by UV lamps submerged in the effluent (Brahmi & Hassen 2011). The adoption of UV light for wastewater disinfection has grown significantly over the past few decades. Indeed, many wastewater management agencies have converted from chemical-based disinfection to UV radiation technology due to its sustainability (Pang *et al.* 2016). Moreover, UV radiation is one of the few cost-effective disinfection alternatives that do not have the potential to create or release carcinogenic by-products into the environment and it is also an effective disinfectant for chlorine-resistant protozoa such as *Cryptosporidium* and *Giardia* (Rizzo *et al.* 2014).

Ultrasound is cyclic sound pressure that has a frequency which is greater than the upper limit of human hearing. It is based on a sound mechanical energy transmitted by pressure waves in a material medium (Farooq *et al.* 2009). This technology has also been adopted in wastewater purification for the elimination of pathogenic microorganisms (Kumar *et al.* 2014). The application of ultrasound in water treatment involves the use of ultrasound systems such as the Sonic systems, which are manufactured to suppress specific microbial growth and biofilm formation (Farooq *et al.* 2009; Kumar *et al.* 2014).

Despite the suitability of the two emerging techniques (UV radiation and US) in sustainably increasing the purification efficiencies of wastewater purification, their application in eliminating faecal pathogenic microorganisms such as *E. coli* has not been fully explored. Moreover, their potential in the elimination of *E. coli* resistant genes are inadequately understood. This study was therefore aimed at evaluating the individual and combined/integrated potential of UV radiation and ultrasonic (US) technologies in the removal of sulfonamide and tetracycline resistant *E. coli* from domestic effluents.

METHODOLOGY

Sampling and membrane filtration technique

Wastewater in this study was sampled twice a month (between the months of March and June 2018) from the effluent of a municipal wastewater treatment plant (WWTP) in Xian, China. The biological treatment of the sampled WWTP was an anoxic/anaerobic process. The raw wastewater had the following characteristics: chemical oxygen demand (COD), 60 mg/L; nitrate, 6 mg/L; nitrite, 0.2 mg/L; ammonium, 1–2.0 mg/L; total suspended solids, 200 mg/L; total nitrogen, 6–8 mg/L; pH, 6.4 and conductivity, 250 mS/m. All the samples were collected using 500 mL sterile containers, stored at 4 °C and ferried to the laboratory under ice conditions for further processing. The water samples were then subjected to membrane filtration techniques (MFT) following the procedures outlined in APHA (2005) and Donde *et al.* (2017). In brief, water samples (100 ml) were filtered through a mixed cellulose ester membrane with a pore size of 0.45 μm and placed onto petri dishes with chromocult agar (Merck) plates and incubated at 37 °C for 18–24 hours. Typical colonies appearing blue were counted as *E. coli* colonies. Numbers of cells were expressed as colony forming units (CFUs)/100 mL and converted to log units.

Isolation of antibiotic resistant *E. coli*

Ten *E. coli* colonies were randomly selected from the mixed cellulose ester membrane and subjected through PCR to detect the presence of antimicrobial resistant genes (*Sul1, tetA* and *tetB*) using the primer details outlined in the supplementary information and following the procedure outlined in Momtaz *et al.* (2012). To further screen and confirm the antibiotic-resistant colonies, the filtered membranes were placed on separate m-FC agar plates with 16 mg/L of sulfonamide and 16 mg/L tetracycline. All the *E. coli* colonies positive for the resistant genes were then cultivated to mid-log phase at 37 °C in 20 mL of nutrient broth. Each culture was centrifuged at 5,000 rpm/min for 15 min and the pellet was washed twice with sterile distilled water. This procedure was repeated to ensure that only pure resistant *E. coli* was obtained and used as test organism. Five hundred mL of wastewater sampled from the domestic WWTP was sterilized at 121 °C for 30 minutes using an airtight pressure heater and then aseptically cooled to room temperature. Two sets of 100 mL of the sterilized wastewater were separately mixed with 16 mg/L of sulfonamide and 16 mg/L tetracycline resistant *E. coli*. Ten mL of each of the pelleted resistant *E. coli* with the bacterial concentration of 3.8 log units for sulfonamide resistant *E. coli* and 4.4 log units for tetracycline resistant *E. coli* were put into separate 250 mL sterile flasks and then separately seeded with 90 mL of sterile primary wastewater giving an initial approximate concentration of 3.8 and 4.4 log units for viable cell counts/100 mL respectively for sulfonamide resistant *E. coli* and tetracycline resistant *E*. The seeded antibiotic-resistant *E. coli* were then subjected to UV and US purification experiments.

Ultraviolet radiation and ultrasonic purification experiments

Wastewater samples of 20 mL with the seeded antibiotic-resistant *E. coli* were placed into transparent 50 mL properly labeled test tubes with screw caps (95% transparent for 360 nm light). Each seeded drug resistant *E. coli* viable counts (sulfonamide and tetracycline resistant *E. coli*) were exposed to three doses (0, 300 and 600 mW s cm^{-2}) of UV radiation that were fixed in a chamber. The sample was continuously mixed gently using a sterile magnetic stir bar. The seeded *E. coli* were then aseptically sampled at different time intervals (0, 30, 60, 120 and 240 min) and CFU quantified using MFT. For each seeded drug resistant *E. coli* tested, a UV radiation experiment was performed in triplicate. The collimated UV light was provided by a 15-W mercury vapor 254-nm lamp which was directed onto the transparent tubes containing the seeded drug resistant *E. coli* within a closed chamber. UV light intensity was measured using a radiometer equipped with a UV 254 detector. All the tests were carried out at *E. coli* optimum growth temperature conditions of 37.5 °C.

For the US experiment, the seeded antibiotic-resistant *E. coli* (sulfonamide and tetracycline resistant *E. coli*) were placed in transparent 50 mL properly labeled test tubes without screw caps and were subjected to different frequencies of US sounds (35 and 130 kHz) under 250 W power. The seeded *E. coli* were then aseptically sampled at different time intervals (0, 30, 60, 120 and 240 min) and CFU quantified using MFT. For each tested seeded drug resistant *E. coli*, US experiments were performed in triplicate.

To evaluate the potential of combined/integrated UV radiation and US wastewater treatment technologies in the eradication of antibiotic resistant *E. coli*, an additional experimental run was set. This involved combined UV radiation and US purification technology set ups, and the samples were subjected to the combined conditions and sampling. The seeded drug resistant *E. coli* viable counts that were subjected to the combined condition were then aseptically sampled at different time intervals (0, 30, 60, 120 and 240 min) and CFU quantified using MFT. For each tested seeded drug resistant *E. coli*, the combined UV radiation-US experiment was performed in triplicate. A summary of UV and US dosage with respective sample identities for different time durations are summarized in Table 1 and detailed information is provided in the supplementary information.

Statistical analysis

Statistical analyses were performed using MINITAB statistical package version 14. The sample size generated under each treatment was 15, normality tests were run for every data set prior to statistical analysis. Mean bacterial colony count values were calculated and presented in log units. Mean comparisons were performed using one-way analysis of variance (ANOVA) at 95% confidence level. Where there was significant difference between the means, the

Table 1 | Sample identities for UV and US dosages on different time duration

		Time (minutes)		
***E. coli* type**	**Dosage**	**0**	**60**	**240**
Sulfonamide resistant	0 mW·s·cm^{-2}	SR-0-0	SR-60-0	SR-240-0
	300 mW·s·cm^{-2}	SR-0-300	SR-60-300	SR-240-300
	600 mW·s·cm^{-2}	SR-0-600	SR-60-600	SR-240-600
Tetracycline resistant	0 mW·s·cm^{-2}	TR-0-0	TR-60-0	TR-240-0
	300 mW·s·cm^{-2}	TR-0-300	TR-60-300	TR-240-300
	600 mW·s·cm^{-2}	TR-0-600	TR-60-600	TR-240-600
Sulfonamide resistant	35 kHz	SR-0-35	SR-60-35	SR-240-35
	130 kHz	SR-0-130	SR-60-130	SR-240-130
Tetracycline resistant	35 kHz	TR-0-35	TR-60-35	TR-240-35
	130 kHz	TR-0-130	TR-60-130	TR-240-130
Sulfonamide resistant	600 mW·s·cm^{-2} and 130 kHz	SR-0-600-130	SR-60-600-130	SR-240-600-130
Tetracycline resistant	600 mW·s·cm^{-2} and 130 kHz	TR-0-130	TR-60-600-130	TR-240-600-130

Tukey test was run as a post-hoc test to determine the points of mean variation. Students *t*-test was used in comparing the mean of *E. coli* resistant genes at the highest dosages and longest time between UV radiation and US treatments.

RESULTS

Ultraviolet radiation purification experiment

The results from the UV radiation experiment for the removal of sulfonamide resistant *E. coli* are presented in Figure 1(I). From an initial value of 3.8 log units of the sulfonamide resistant *E. coli,* there was a reduction to a final value of 0.8 log unit at 240 min and UV dosage of 600 mW s cm^{-2} (SR-240-600). There was statistical difference in colony counts of sulfonamide resistant *E. coli* between the different UV radiation dosages ($P < 0.05$). The Tukey test showed a lack of statistical difference in the mean colony counts of sulfonamide resistant *E. coli* between 240 min at 300 mW s cm^{-2} UV dosage (SR-240-300) and 60 min at 600 mW s cm^{-2} UV dosage (SR-60-600) treatments ($P > 0.05$). The results on the UV radiation experiment for the removal of tetracycline resistant *E. coli* are presented

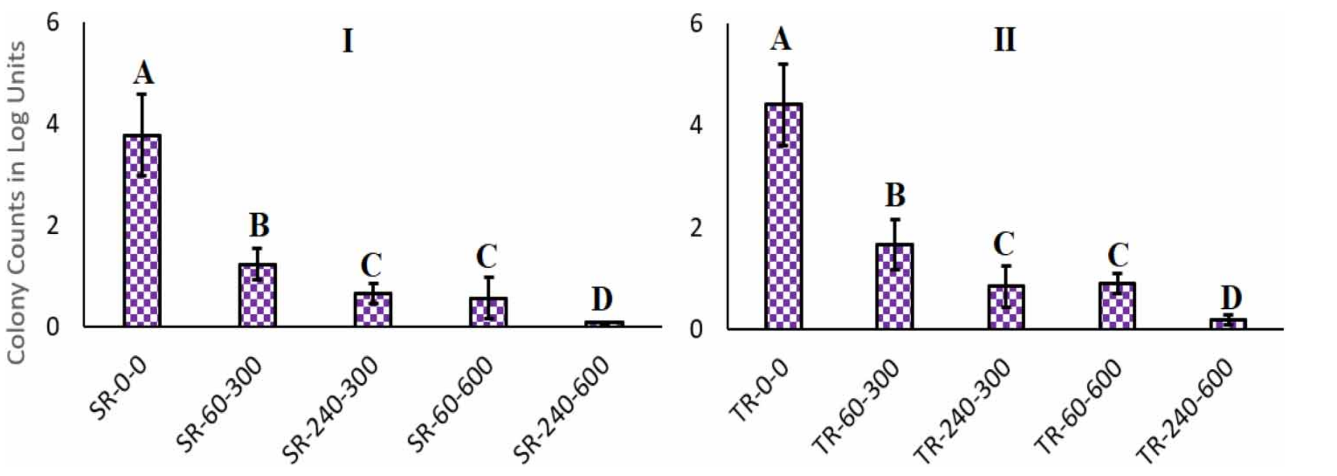

Figure 1 | Colony counts for (I) sulfonamide resistant *E. coli* at different UV radiation dosages for different time duration and (II) for tetracycline resistant *E. coli* at different UV radiation dosages for different time durations ($N = 15$, $P \leq 0.05$).

in Figure 1(II). From an initial value of 4.4 log units of the tetracycline resistant *E. coli*, there was a reduction to a final value of 2 log units at 240 min and UV dosage of 600 mW s cm^{-2} (SR-240-600). There was statistical difference in the mean log units of tetracycline resistant *E. coli* between the different UV radiation dosages ($P < 0.05$). The Tukey test showed a lack of statistical difference in the mean values of tetracycline resistant *E. coli* between 240 min at 300 mW s cm^{-2} UV dosage (SR-240-300) and 60 min at 600 mW s cm^{-2} UV dosage (SR-60-600) treatments ($P > 0.05$).

Ultrasonic purification experiments

The results of the US experiment for the removal of sulfonamide resistant *E. coli* are presented in Figure 2(I). From an initial value of 3.8 log units of the sulfonamide resistant *E. coli*, there was a final reduction value of 0.3 log units CFU at 240 min and US dosage of 130 kHz (SR-240-130). There was statistical difference in the mean of log units of sulfonamide resistant *E. coli* between the different US dosages ($P < 0.05$). The results from the US experiment for the removal of tetracycline resistant *E. coli* are presented in Figure 2(II). From an initial value of 4.4 log units of the tetracycline resistant *E. coli*, there was a reduction to a final value of 0.6 log units at 240 min and US dosage of 130 kHz (SR-240-130). There was statistical difference in the mean CFU of sulfonamide resistant *E. coli* between the different US dosages ($P < 0.05$). The mean CFU of sulfonamide resistant *E. coli* was significantly higher at US dosage of 130 kHz for 60 min than that at US dosage of 35 kHz for 240 min.

Comparison between UV radiation and US purification experiments

Results on the comparison between UV radiation and US experiments in the removal of sulfonamide resistant *E. coli* is provided in Figure 3(I). There was a significant statistical difference between the 300 mW s cm^{-2} UV for 60 min of UV dosage (SR-60-300) and the 35 kHz for 60 min of US dosage (SR-60-35); between the 300 mW s cm^{-2} for 60 min UV dosage (SR-240-300) and the 35 kHz for 240 min of US dosage (SR-240-35); between the 600 mW s cm^{-2} for 240 min UV dosage (SR-240-600) and the 130 kHz for 240 min of US dosage (SR-240-130) ($P < 0.05$). However, there was a lack of significance between the 600 mW s cm^{-2} UV for 60 min of UV dosage (SR-60-600) and 130 kHz for 60 min of US dosage (SR-60-130) ($P > 0.05$).

Results on the comparison between UV radiation and US experiments in the removal of tetracycline resistant *E. coli* is provided in Figure 3(II). There was significant statistical difference between the 300 mW s cm^{-2} UV for 60 min of UV dosage (TR-60-300) and the 35 kHz for 60 min of US dosage (TR-60-35); between the 300 mW s cm^{-2} UV for 240 min of UV dosage (TR-240-300) and the 35 kHz for 240 min of US dosage (TR-240-35); between the 600 mW s cm^{-2} UV for 60 min of UV dosage (TR-60-600) and the 130 kHz for 60 min of US dosage (SR-60-130); between

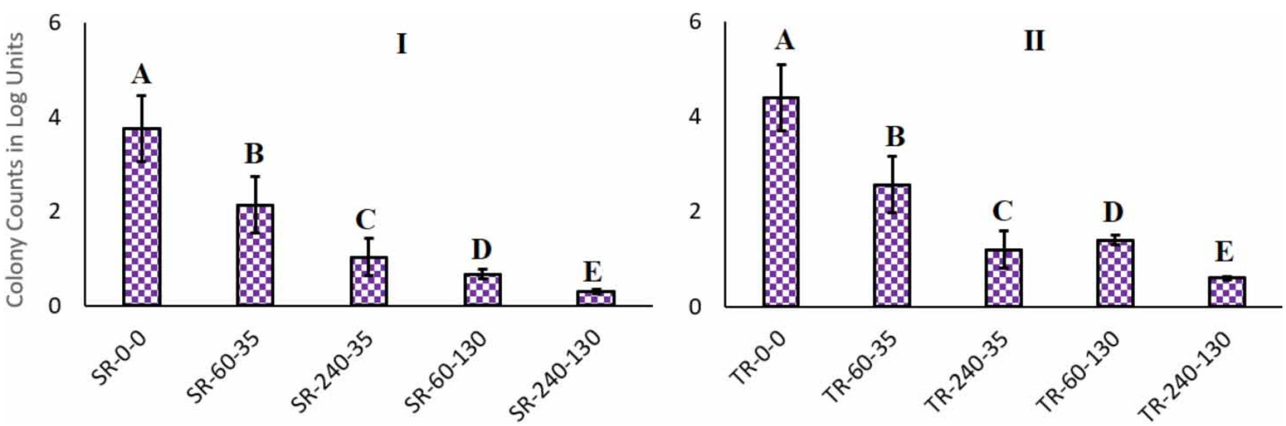

Figure 2 | Colony counts for (I) sulfonamide resistant *E. coli* at different US dosages for different time duration and (II) for tetracycline resistant *E. coli* at different US dosages for different time durations ($N = 15$, $P \leq 0.05$).

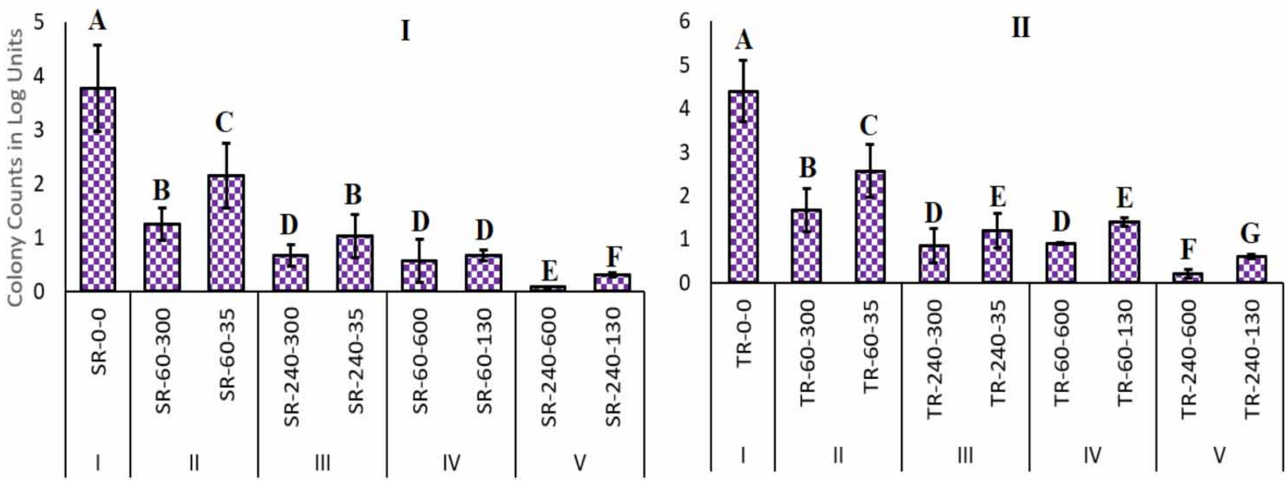

Figure 3 | Colony counts for (I) sulfonamide resistant *E. coli* at different UV radiation and US dosages for different time duration and (II) for tetracycline resistant *E. coli* at different UV radiation and US dosages for different time durations ($N = 15$, $P \leq 0.05$).

the 600 mW s cm^{-2} UV for 240 min of UV dosage (TR-240-600) and the 130 kHz for 240 min of US dosage (TR-240-130) ($P < 0.05$).

Combined UV radiation and US purification experiments

Results on combined UV radiation and US experiments for the removal of sulfonamide resistant *E. coli* is presented in Figure 4(I). From an initial value of 3.8 log units of the sulfonamide resistant *E. coli*, there was a reduction to a final value of 0.005 log units at UV radiation dosage of 600 mW s cm^{-2} and US dosage of 130 kHz for 240 min (SR-240-600-130).

Results on combined UV radiation and US experiments for the removal of tetracycline resistant *E. coli* are presented in Figure 4(II). From an initial value of 4.4 log units of the tetracycline resistant *E. coli*, there was a reduction to a final value of 0.002 log units at UV radiation dosage of 600 mW s cm^{-2} and US dosage of 130 kHz for 240 min (SR-240-600-130).

DISCUSSION

The availability of safe, clean drinking water has remained a challenge, not only in the less developed countries but also

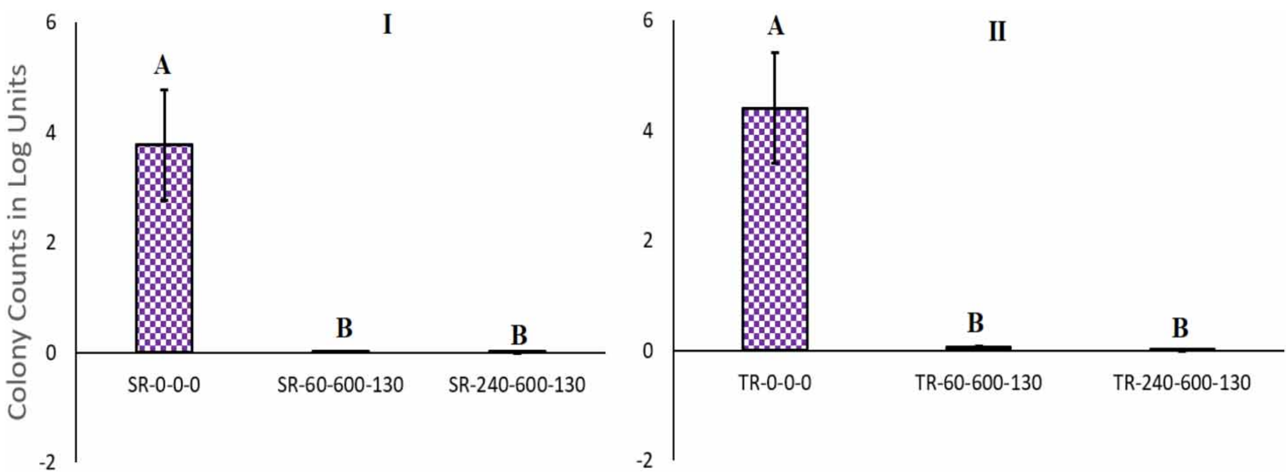

Figure 4 | Colony counts for (I) sulfonamide resistant *E. coli* at combined UV radiation and US dosages for different time duration and (II) for tetracycline resistant *E. coli* at combined UV radiation and US dosages for different time durations ($N = 15$, $P \leq 0.05$).

in the developed world, due to the rise in wastewater-related resistant genes. The crisis is currently global, and no region or country is considered safe from the emerging resistant genes (Schijven *et al.* 2019). Pathogens in wastewater can be inactivated or destroyed by either chemical or physical processes (Okoh & Igbinosa 2010). UV radiation can not only kill the bacteria present, but also disrupt bacterial reproduction which can inhibit cell growth and induce gene damage. Indeed, bacterial pathogens on various media respond differently to UV light exposure, and this highly depends on the physical characteristics of the medium (Adhikari *et al.* 2015). The major limitation of UV radiation technology is lack of enough solar radiation, a situation not common in tropical regions. A high level of suspended solids may also hinder the effectiveness of both the UV radiation and US wastewater purification technologies, hence technological integration is recommended (Donde *et al.* 2015). Indeed, various forms of UV such as UV–TiO_2 photocatalysis technology has been shown to be a promising non-chemical and residue-free method with reduced water usage and it is more environmentally friendly for ensuring microbiological safety and maintaining the nutritional quality of fresh blueberries and other fresh produce during postharvest processing (Mijin *et al.* 2018). Different studies have provided contradicting findings on the application of UV radiation in the removal of antibiotic resistant genes. Zhang *et al.* (2017) showed that UV disinfection could not effectively eliminate antibiotic resistant genes (ARGs). In contrast, the study by Guo *et al.* (2013) indicated that a UV dose of 10 mJ cm^{-2} could effectively eliminate erythromycin resistance genes.

Complete removal of *E. coli* resistant genes can be beneficial in lowering the load of ARGs from aquatic systems. Indeed, the risk of the spread of antibiotic resistance to downstream microbial communities still exists. This is because of the persistent antibiotic resistant genes that tend to reoccur after the treatment process. Such genes can further be consequently captured and transferred to other organisms such as pathogenic or indicator microorganisms, resulting in challenges in the control of waterborne and other water related diseases. Therefore, effective and more advanced disinfection methods need to be selected to minimize the occurrence, spread and accumulation of the antibiotic resistant genes (Guo *et al.* 2017). The study showed a remarkable removal of the sulfonamide resistant *E. coli* at 240 min and UV dosage of 600 mW s cm^{-2}. Indeed, the statistical difference in the mean CFU of sulfonamide resistant *E. coli* between the different UV radiation dosages ($P < 0.05$) was an indication of the removal different potential at varying dosages and time durations. There was a lack of significant statistical difference in the mean values of sulfonamide resistant *E. coli* at 300 mW s cm^{-2} UV dosage for 240 min (SR-240-300) and at 600 mW s cm^{-2} UV for 60 min dosage (SR-60-600) treatments. This showed that lower UV radiation dosages require more time to effectively remove the antibiotic resistant *E. coli*. Therefore, time duration should be a crucial consideration in the eradication of the antibiotic resistant genes from the water bodies, a situation that has increased the health risk due to the rise in horizontal gene transfer and spread within the aquatic ecosystems (Huddleston 2014). Additionally, the study equally showed a remarkable removal of tetracycline resistant *E. coli* at UV dosage of 600 mW s cm^{-2} for 240 min. This proved that the UV radiation had great potential in the removal of multidrug resistant genes. Indeed, such a finding may offer an alternative to that proposed by Zeinab *et al.* (2010), where a low dose of gamma radiation had been applied in the removal of multidrug resistant pathogens.

Purification efforts have equally been shifting focus to the use of underwater shock waves on living cells. Ultrasonication is one of the very useful techniques for the treatment of wastewater and it is capable of decreasing bacterial population in wastewater. Through this technique, as the frequency and time period increases, the bacterial population also decreases. Indeed, the destruction effect of US waves on microorganisms, with emphasis on the pathogenic group, is still being improved upon (Tiwari *et al.* 2008). Specifically, studies have considered the bactericidal effect of US waves on different *E. coli* species. Some of these findings have indicated that US is capable of producing pressure variation, cavitation and the radiation resulting from the underwater shock wave, which has the ability to reduce the viability of these microorganisms (Kumar *et al.* 2014). The findings from the current study showed the high potential of US radiation in the removal of sulfonamide resistant *E. coli* from an initial value of 38,000 CFU of the sulfonamide resistant *E. coli*, to a final reduction value of

3,000 CFU at 240 min and US dosage of 130 kHz. Additionally, it showed the removal variation by different dosages of US sounds at varying time durations. There was statistical difference in the mean CFU of sulfonamide resistant *E. coli* between the different US dosages ($P < 0.05$). The mean CFU of sulfonamide resistant *E. coli* was significantly higher at US dosage of 130 kHz for 60 min than at US dosage of 35 kHz for 240 min. Moreover, the technology was also capable of lowering the CFU of tetracycline resistant *E. coli* from an initial value of 4.4 log units of the tetracycline resistant *E. coli* to a final reduction value of 0.6 log units at 240 min and US dosage of 130 kHz. Furthermore, the study confirmed that higher dosages take shorter time durations to achieve lower values of both the sulfonamide and tetracycline resistant *E. coli*.

Like UV radiation, US has been used in wastewater purification. Even though UV radiation has proven more effective than US in eliminating microorganisms within a clearer wastewater, its stand-alone application is still discouraged as combining it with US may help solve wastewater color-related limitations (Khoobdel *et al.* 2010). The present study indicated that UV radiation technology was more effective in the removal of both sulfonamide and tetracycline resistant *E. coli* from wastewater. Sonication effects have been shown to increase the UV disinfection efficiency in terms of the reduction of large particles and cleaning of the bacterial lamps (Naddeo *et al.* 2009; Doosti *et al.* 2012). Indeed, the combined process of UV radiation-US techniques can be considered as a valuable alternative to conventional oxidation/disinfection processes. Therefore, the advanced UV radiation-US technique, applied under such conditions, may be an effective technique in all WWTP where wastewater reuse is an important integrative/alternative resource for non-drinking purposes. From the present study, the combined UV radiation-US technique improved the removal of both the sulfonamide and tetracycline resistant *E. coli* to CFUs of below 30 per 100 mL of wastewater. This was an improvement in the removal of the sulfonamide and tetracycline resistant *E. coli* from the single techniques (separately operating UV radiation technique or US technique). This may be due to the limitations that exist when a single technique is used. For example, single UV radiation or US wastewater treatment technologies may be limited to the nature of particles in the wastewater as the water to be treated must not be too turbid or cloudy (Khoobdel *et al.* 2010; Gibson *et al.* 2017; EL-Shahawy *et al.* 2018).

CONCLUSIONS

Even though UV radiation has proven more effective than US in eliminating microorganisms within a clearer wastewater, its stand-alone application is still not 100% effective and combining it with US enhances the treatment procedure by solving treatment limitations, such as the color of the wastewater. The present study showed that UV radiation technology was more effective in the removal of both sulfonamide and tetracycline resistant *E. coli* from wastewater. However, the integrated UV radiation-US technique was highly efficient and is recommended in the removal of antibiotic resistant *E. coli* from wastewater. Nonetheless, further studies also need to be performed to better evaluate the disinfection effectiveness on different bacteria species and also using different wastewater with different types and quantities of pollutants.

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SUPPLEMENTARY MATERIAL

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