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Synergistic bactericidal activity of ultraviolet radiation, ozone, and liquid-thin-film technology against *Escherichia coli* in water

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ABSTRACT

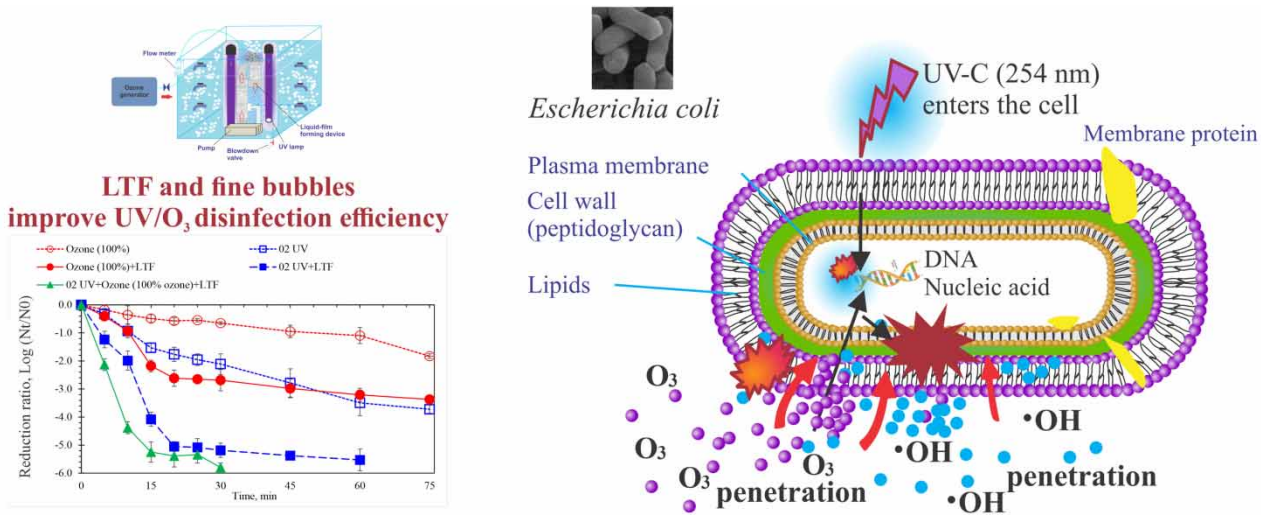
Providing effective and sustainable water disinfection methods, without harmful by-products, is essential to protect public health and safety. It is hypothesized that the application of liquid-thin-film (LTF) technology can enhance the bactericidal activity of both ultraviolet (UV) irradiation and ozone (O_3) treatments. Therefore, this study aimed to examine the bactericidal activity and synergistic effect of the combined UV + O_3 + LTF treatment against *Escherichia coli* in water processing. The results showed that LTF technology significantly increased the disinfection efficiency of UV and O_3 in potable water production. Thus, the combined UV + LTF, O_3 + LTF, and UV + O_3 + LTF treatments were more effective than the individual treatments under identical conditions. Particularly, the combined double-UV (41.7 mJ cm^{-2}) + O_3 (100% O_3) + LTF (65 L min^{-1}) treatment exhibited the highest bactericidal activity, resulting in a 5.8-log decrease in the *E. coli* load within 30 min. Pearson's correlation analysis demonstrated a significant correlation between the synergistic effects of the combined treatments and UV dosage ($r = 0.88$, $p < 0.0001$) and aqueous O_3 concentrations ($r = 0.84$, $p < 0.0001$). These findings suggest that combined UV + O_3 + LTF treatment could be a viable alternative method for water disinfection.

Key words: *Escherichia coli*, liquid-thin-film, ozone, synergy, UV radiation, water disinfection

HIGHLIGHTS

- Liquid-thin-film (LTF) enhances the bactericidal activity of UV rays and O_3 disinfectant.
- The combined UV radiation, ozone, and LTF had synergistic bactericidal effects.
- UV + O_3 + LTF combined treatment had the highest bactericidal activity against *E. coli*.
- *E. coli* were susceptible to UV + O_3 + LTF at a low chemical dosage and short exposure time.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Currently, ~844 million people lack access to clean water, particularly residents of developing countries (WHO and UNICEF 2017). In Vietnam and most developing countries, large amounts of untreated domestic wastewater are discharged into natural water bodies, which is an environmental and health risk. There has been an increase in the incidence of water-borne diseases, such as cholera, typhoid fever, dysentery, hepatitis A, and intestinal infections, owing to the contamination of drinking water by pathogenic microbes, such as *Vibrio cholera*, *Salmonella typhi*, *Shigella* spp., *Ascaris lumbricoides*, *Cryptosporidium parvum*, *Schistosoma*, and enteropathogenic *Escherichia coli* (WHO and UNICEF 2017). Therefore, removing potentially harmful chemical, microbiological, or physical contaminants through water treatment techniques is essential to improve the quality of drinking water. Conventionally, water is treated using a multibarrier treatment process consisting of pre-oxidation, flocculation, sedimentation, filtration, and disinfection. Disinfection is the final step in drinking water processing to prevent water-borne disease transmission.

Presently, there are emerging concerns regarding disinfection methods for drinking water treatment. An ideal disinfection method should be inexpensive, simple to implement, and able to rapidly and effectively eliminate numerous microbes without generating harmful by-products. Conventional disinfection methods include chemical and physical treatment. Although chemical disinfection remains a widely investigated approach, the application of this method may have problems related to the generation of potentially harmful disinfection by-products (DBPs). For example, chlorination is a widely used method for water and wastewater disinfection; however, this method may produce potential carcinogenic agents, such as trihalomethanes (THMs), haloacetic acids (HAAs), and bromate (Boorman *et al.* 1999; Srivastav *et al.* 2020). Compared with chlorine (standard redox potential $E^0 = 1.36$ V/NHE), ozone (O_3) is a strong oxidizing agent ($E^0 = 2.07$ V/NHE). Ozone can effectively inactivate several microbes, including bacteria, viruses, spores, cysts, and *Giardia,* and *Cryptosporidium* cysts (Cho *et al.* 2003; Von Gunten 2003; Pichel *et al.* 2019), making it a suitable alternative to chlorination. In water disinfection, the normal concentration of ozone application is in the range of 5–10 mg L^{-1} (Huang *et al.* 2003; Blatchley *et al.* 2012). However, ozone must be produced on-site owing to its instability and low solubility in water (14 mmol L^{-1} at 20 °C), and it is difficult to determine the necessary ozone oxidizing dose (Cho *et al.* 2003). Hence, this method is expensive and has high energy requirements for industrial applications. Additionally, although high ozone doses can improve disinfection efficiency, they can also promote the formation of DBPs, especially bromate formation in water containing bromide (Huang *et al.* 2003; Von Gunten 2003). Recently, physical disinfection methods, such as ultrasound, ultra-high pressure, high-voltage pulsed electric fields, plasma technology, cavitation, and UV radiation, have attracted increasing research attention for water treatment (Tsolaki & Diamadopoulos 2010; Kheyrandish *et al.* 2017; Pichel *et al.* 2019; Gorito *et al.* 2021). Although physical disinfection does not generate residual toxicity, these methods have substantial energy consumption and high operating costs (Tsolaki & Diamadopoulos 2010). Therefore, it is important to develop advanced and sustainable methods for water disinfection.

To date, several studies have evaluated the possibility of combining different water disinfection methods to improve water quality and obtain synergistic benefits (Lubello *et al.* 2002; Koivunen & Heinonen-Tanski 2005; Magbanua *et al.* 2006; Jung *et al.* 2008; Blatchley *et al.* 2012; Dang *et al.* 2016; Giannakis *et al.* 2016; Gorito *et al.* 2021); one such potential approach is the combination of ultraviolet (UV) radiation and O_3 treatment.

UV radiation has been extensively applied in food sterilization and water disinfection because of its broad-spectrum disinfectant ability and non-residual toxicity (Hijnen *et al.* 2006; Bowker *et al.* 2011; Kheyrandish *et al.* 2017). However, the implementation of UV is limited because of the presence of UV-absorbing compounds and particles in water, which inhibit UV light transmittance into bacterial cells, thereby preventing total bacterial inactivation (Tsolaki & Diamadopoulos 2010; Farrell *et al.* 2018). An alternative approach is the combined application of UV light and oxidants, such as O_3, peracetic acid (PAA), chlorine, and hydrogen peroxide (H_2O_2) (Lubello *et al.* 2002; Koivunen & Heinonen-Tanski 2005; Giannakis *et al.* 2016). Lubello *et al.* (2002) reported that the combined PAA (2 mg L^{-1})/UV (120 mW s cm^{-2}) treatment method achieved improved disinfection efficiency with 3.6-log reduction in *E. coli* load, whereas only 2.8-log and 3.0-log reduction, respectively, were achieved by the two individual treatments (PAA and UV). In contrast, combined H_2O_2/UV (120 mW s cm^{-2}) disinfection at low H_2O_2 dosages did not significantly enhance UV effectiveness; only at high H_2O_2 dosages (>20 mg L^{-1}), a slight increase in disinfection efficiency (~1 log) was observed by the combined H_2O_2/UV treatment (Lubello *et al.* 2002). Koivunen & Heinonen-Tanski (2005) studied the disinfection efficiencies of PAA, H_2O_2, and sodium hypochlorite (NaOCl) against *E. coli*, *Enterococcus faecalis*, *Salmonella enteritidis,* and coliphage MS2 viruses and found that combined PAA/UV disinfection achieved significantly higher bactericidal activity than other methods. Additionally, Giannakis *et al.* (2016) observed an increase in the disinfection efficiency of UV irradiation with the addition of H_2O_2. Despite the increased disinfection effect of the combined treatments, the use of high-dose chemical agents may induce the generation of DBPs.

Recently, liquid-thin-film (LTF) technology, which involves a liquid-film-forming apparatus (LFFA), has been extensively studied in water and wastewater treatment and various other applications because of its specific properties (Imai & Zhu 2011; Vo *et al.* 2014; Dang *et al.* 2016; Imai & Dang 2017; Nguyen *et al.* 2018). LTF is known to enhance the contact area between gas and water, provide relatively long-term durability in water, and facilitate gas dissolution in water (Imai & Zhu 2011; Nguyen *et al.* 2018). A number of studies have reported that the application of LTF can improve water disinfection. For instance, Vo *et al.* (2014) reported that an LTF of pressurized carbon dioxide (CO_2) at 0.7 MPa and room temperature inactivated more than 3.3-log bacteriophage Qβ and ~3.0-log bacteriophage ΦX174 within 25 min. Imai & Dang (2017) employed an LTF of pressurized CO_2 in the treatment of sea ballast water and succeeded in eliminating *E. coli* within 3 min under identical treatment conditions (0.7 MPa, 20 °C, 25 L min^{-1}, 50% WVR, $\Delta P = 0.12$ MPa, and 15 cycles of pressure cycling). Dang *et al.* (2016) reported that using an LTF of pressurized CO_2 and sodium hypochlorite showed efficient performance in the inactivation of *Enterococcus* sp. in seawater. Additionally, Dang *et al.* (2020) found that the use of the combined LTF (2,400 L min^{-1}) and UV (4.53×10^{-18} mJ cm^{-2}) resulted in a ~95% reduction in *E. coli* population within 75 min, whereas only 32% of the *E. coli* load was reduced by UV treatment alone at the same UV dosage. Though LTF has greatly improved the bactericidal activity of UV light; with the design of a non-submerged UV lamp system, low UV dosage (i.e., 4.53×10^{-18} mJ cm^{-2}), and high flow rate (i.e., 2,400 L min^{-1}), the disinfection efficiency remains low and has not fulfilled the requirement of National Technical Regulation QCVN 01-1:2018/BYT on the quality of water supplied for domestic purposes (*E. coli* level should not exceed 1 CFU 100 mL^{-1}) (Dang *et al.* 2020). Studies are yet to examine whether using LTF combined with UV and a small amount of O_3 in water treatment could improve disinfection efficiency.

It is hypothesized that the use of LTF can substantially improve the solubilization of ozone in water, thereby, enhancing its bactericidal activity. Additionally, UV radiation at 254 nm may induce the formation of free radicals (i.e., •OH; $E^0 =$ 2.8 V/NHE) from photolytic ozone decomposition, which could further enhance the bactericidal effect of the treatments (Chin & Bérubé 2005; Jung *et al.* 2008). Furthermore, it is expected that an increase in the interfacial contact efficiency between gas and water caused by LFFA may help increase UV ray scattering. The bactericidal effect of UV is relatively dependent on UV transmittance. Therefore, the combination of UV and LTF may promote UV activity and increase its bacterial inactivation efficiency (Dang *et al.* 2020). Chin & Bérubé (2005) reported that a combined O_3/UV advanced oxidation process reduced the DBPs' formation potentials (~80% THMs and 70% HAAs). However, since limited information is available on the effect of LTF technology on the bactericidal performance of combined UV/O_3 treatments, it is unclear whether combined UV/O_3 treatment could be an effective primary disinfectant.

Therefore, this study aimed to evaluate the bactericidal efficacy and synergistic effects of combined UV radiation, O_3, and LTF treatment against *E. coli* in contaminated water. The sensitivity of the bacteria to the combined UV + O_3 + LTF treatment was investigated under various UV dosages and ozone supply rates. Additionally, the bactericidal effects of UV radiation and O_3 were examined and compared in both combination and individual treatments, with and without LTF technology.

2. MATERIALS AND METHODS

2.1. Microorganism preparation

The *E. coli* (ATCC 11303) inoculum was prepared by inoculating the bacterial stock (American Type Culture Collection, Manassas, VA, USA) in 100 mL of Luria-Bertani (LB) broth (Wako Chemical Co. Ltd, Osaka, Japan). The *E. coli* culture was incubated at 37 °C with shaking at 150 rpm for 20 h, and the permanent stock was preserved in 30% glycerol at –60 °C.

For each disinfection experiment, 100 μL of *E. coli* culture glycerol stock was transferred to 100 mL of LB broth and incubated at 37 °C under continuous shaking at 150 rpm for 20 h. Bacterial biomass was then centrifuged for harvesting, and rinsed with physiological saline solution three times (10 min at 10,000 rpm at 4 °C) using a refrigerated centrifuge (Allegra X-30R, Beckman Coulter, Inc, USA). The pellet was then re-suspended in saline solution. The *E. coli* culture was immediately used to prepare artificial micro-polluted water samples.

2.2. Preparation of artificial micro-pollution water sample

Disinfection experiments were performed using an artificial micro-polluted water sample. The water sample was prepared by adding the bacterial culture to tap water. Tap water was obtained from the local water supply of Hue City (HueWACO Co., Ltd). The basic water quality parameters of the tap water were as follows: total iron, 0.01 mg L^{-1}; Mn, 0.001 mg L^{-1}; turbidity, 0.02–0.1 NTU; pH, 7.0–7.5; hardness, 22 mg $CaCO_3$ L^{-1}; nitrite, 0.003 mg N-NO_2 L^{-1}; and residual chlorine, 0.5–0.6 mg L^{-1}. For all experiments, a 0.02 M solution of sodium thiosulfate pentahydrate ($Na_2S_2O_3{\cdot}5H_2O$; Wako, Japan) was added to the tap water to completely quench residual chlorine. The *N,N*-diethyl-*p*-phenylenediamine (DPD; HI701-0 free chlorine reagent, Hanna Instruments SRL, Romania) colorimetric method with an ion-specific meter (HI701, Hanna Instruments SRL, Romania) was employed for chlorine content determination (to confirm residual chlorine disposal). The samples were continuously aerated for 60 min under UV irradiation to completely remove the remaining thiosulfate (Ahmad *et al.* 2015). Thereafter, the prepared *E. coli* culture was added to the water sample to achieve an initial bacterial concentration of 5–6 $\log_{10}$ CFU mL^{-1}. The dissolved oxygen (DO) and temperature of the samples were measured using a DO meter (Pro 2030, YSI Incorporated, USA).

The aqueous ozone concentration of the samples was immediately measured using the DPD colorimetric method (HI 93757-0 ozone reagent, Hanna Instruments SRL, Romania) with a checker disc 38054 (HI38054, Hanna Instruments SRL, Romania) in the range of 0–2.3 mg L^{-1} with the smallest increment of 0.1 mg L^{-1}. The gas ozone concentration produced by the ozone generator equipment was detected using the double-beam UV absorption method (UVO3-2000S, Shenzhen O3 Tech Co., Ltd, China) with an ozone analyzer flow (RS 485, Shenzhen O3 Tech Co., Ltd, China).

2.3. Microorganism enumeration

E. coli colonies were evaluated using the spread-plate technique. Briefly, a series of 10-fold dilutions were prepared using sterile saline (0.85% NaCl), and 100 μL of either diluted or undiluted samples was plated on the surface of Chromocult® Coliform media plates (Merck & Co., Inc., Darmstadt, Germany). The inoculated medium was incubated for 24 h at 37 °C. Dark blue colonies suspected to be *E. coli* were counted on each plate containing 25–300 CFU, and data were reported as the mean number of CFU mL^{-1}. In the case of undiluted samples with less than 30 CFU/plate, the *E. coli* colonies were counted using the pour technique (1 mL of the sample was poured into coliform agar, which was maintained at 45 °C). After incubating the plates for 24 h at 37 °C, the dark blue colonies were counted. Each sample was examined three times.

2.4. Apparatus and procedure for UV, UV + LTF and UV + O_3 + LTF disinfection experiments

The pilot apparatus was a clear acrylic chamber with an internal volume of 130 L (B × H × L: 48 × 55 × 50 cm); and the setup is shown in Figure 1. The disinfection reactor was designed to include a LFFA (FB-50 h, Ube Kogyo K.K., Japan) to generate numerous fine bubbles and the LTF and to facilitate water movement. The LFFA (W × H × L = 200 × 300 × 80 mm) had extremely low energy consumption; particularly, a single FB-50 h unit could move and aerate water at 20 L min^{-1} with 20 W. A diffuser (30 Ø), quarter-inch airline coupler (hose OD 10 Ø × ID 6.5 Ø), and urethane turbine were set up inside

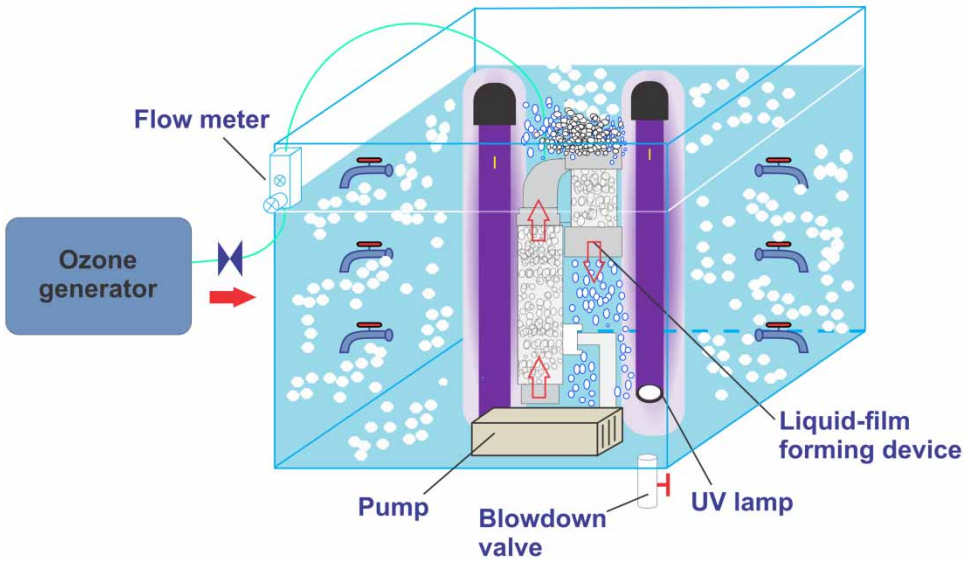


Figure 1 | Experimental setup of the water disinfection apparatus.

the LFFA to create the LTF. Water entered the LFFA from the bottom using a water pump (150 W, AP5400, LifeTech, Guangdong Zhenhua Electrical Appliance Co., Ltd, China); gas was also introduced at the bottom of LFFA through the diffuser and airline coupler at a flow rate of 1.0 L min^{-1}. Thereafter, the water moved to the top of the LFFA and then returned to the bottom of the apparatus, allowing it to move and aerate water simultaneously. During the disinfection process, a large number of LTFs formed, which substantially enhanced the gas–water interfacial area (Figure 1). Ozone was generated using an ozone generator (Jetek, Huetronics JSC., Vietnam), which could be adjusted to different O_3 supply rates (25, 50, 75, and 100%) with an O_3 output of 20 g h^{-1}. The airflow rate was measured using a flow meter (RK-1250, KOFLOC Co., Ltd, Japan). UV lamps (low pressure, 254 nm, Fort DI-55 W, China) were installed inside the apparatus and were submerged in water. The disinfection experiments were conducted in batch mode.

In the combined UV + LTF disinfection experiments, 105 L of water was introduced into the apparatus, and the UV lamp system and LFFA were simultaneously switched on. The water pump (150 W, AP5400, LifeTech, Guangdong Zhenhua Electrical Appliance Co., Ltd, China), which was connected to the LFFA (FB-50h, Japan), was operated at a flow rate of 65 L min^{-1}. During a treatment period of 75 min, the UV intensities were 20.8 and 41.7 mJ cm^{-2}, corresponding to one and two UV lamps (single-UV and double-UV), respectively. For UV treatment alone, the LFFA and pump were removed from the experimental apparatus.

In the experimental setup for ozone application, 105 L of sample water was fed into the apparatus. Ozone was then introduced into the water by the ozone generator (at a gas flow rate of 1.0 L min^{-1}) through the outlet tube to a stone diffuser (19 mm × 30 mm, Youmo Aquapure, China) to create ordinary ozone bubbles in the water. Disinfection was conducted at various ozone supply ratios (25% O_3 + 75% air, 50% O_3 + 50% air, 75% O_3 + 25% air, and 100% O_3) for 75 min.

In the combined O_3 + LTF treatments, ozone gas was fed into the fluid at a flow rate of 1.0 L min^{-1}. The O_3 generator was connected to an LFFA (FB-50h, Japan) at a water flow rate of 65 L min^{-1} to create fine ozone bubbles and LTF. The sensitivity of bacteria to combined O_3 + LTF treatments was determined at various ozone supply ratios (25, 50, 75, and 100%), which were applied for 75 min.

In the combined UV + O_3 + LTF treatments, an appropriate ozone dosage was fed into the reactor via the LFFA to produce fine O_3 bubbles and LTF and to facilitate water circulation. The UV lamp system was started at the same time as O_3 + LTF. The remaining experiments followed the combined O_3 + LTF method described above. To investigate regrowth, the treated samples were analyzed after 3 d of storage in the dark at room temperature (25.3–25.5 °C), and the reactivation potential was determined using the plating method.

Treated water was collected from the six valves of the reactor at different timepoints (0, 5, 10, 15, 20, 25, 30, 45, 60, and 75 min) (Figure 1). The bacterial concentrations were determined as described above. The experiments were repeated thrice.

2.5. Presentation of results

Disinfection efficiency was assessed by the $\log_{10}$ of the reduction ratio from the colony number before and after disinfection. The synergistic bactericidal effect of the combined UV + O_3 + LTF treatment was calculated using the following formula (Dang *et al.* 2016):

Synergy value (log units) = log reduction caused by the combined UV + O_3 + LTF – (log reduction caused by UV alone + log reduction caused by O_3 alone + log reduction caused by LTF alone).

Following this formula, a positive value indicates synergistic benefit because the combined treatment efficiency is greater than the summed efficiency of the individual treatments. In contrast, a negative value indicates antagonism. A zero value indicates no synergy because the combined treatment efficiency is equal to the summed efficiency of the individual treatments.

2.6. Statistical analysis

All statistical analyses were performed using the R program (version 4.0.5, http://cran.R-project.org). Pearson's correlation coefficient was performed to access the relationship between synergy values and other variables such as UV dosage and dissolved O_3 concentrations, and statistical significance was set at 5% ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Bactericidal activity of the UV treatment and combined UV + LTF treatment under different UV dosages

E. coli inactivation was evaluated at two UV radiation doses (one and two UV lamps) and LTF (flow rate of 65 L min^{-1}) in both the UV treatment alone and the combined UV + LTF treatment for 75 min (Figure 2). There was a significant increase in the disinfection efficiency with increasing UV dosage ($R^2 = 0.92$, $p < 2.2e\text{-}16$); however, the bactericidal activity of the combined UV + LTF treatment was significantly higher than that of the UV treatment alone. Additionally, the combined UV + LTF treatment significantly increased the DO levels of the treated water from 6.9 to 8.2 mg L^{-1}, reaching a saturation value at 10 min compared with UV irradiation alone.

The results of the present study showed that the combined UV + LTF treatment yielded significantly higher *E. coli* inactivation efficiency than UV irradiation alone at all UV doses (Figure 2). Approximately 2.5-log and 3.5-log decreases in the *E. coli* count were obtained within 75 min at equivalent UV dosages of 20.8 and 41.7 mJ cm^{-2}, respectively. However, combined single-UV + LTF treatment caused an approximately 3.6-log decrease in the *E. coli* count within 75 min. Additionally, the highest decrease in the bacterial load (>5.5 log) was achieved by the double-UV + LTF combined treatment, resulting in complete inactivation of *E. coli* within 60 min. In contrast, *E. coli* was not inactivated by LTF (65 L min^{-1}) in the absence of UV light. These results indicated that LTF significantly enhanced the UV disinfection efficacy. Particularly, the combined UV + LTF treatments yielded synergistic benefits. Pearson's correlation analysis indicated a significant positive correlation

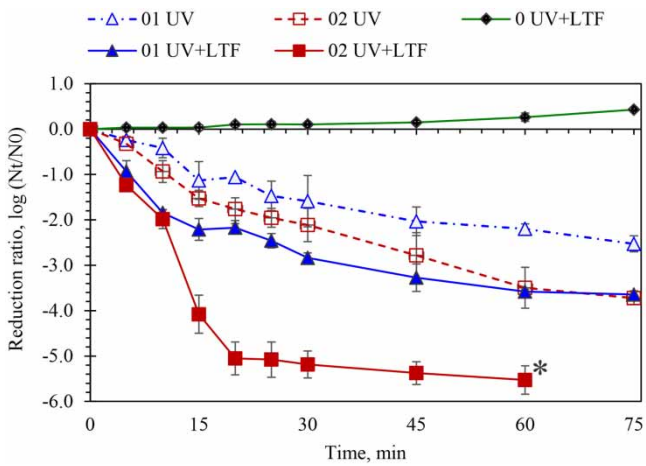


Figure 2 | Comparison of the bactericidal performance of the UV treatment and the combined UV + LTF (flow rate of 65 L min^{-1}) treatment with the effect of various UV dosages (UV dosages = 20.8–41.7 mJ cm^{-2}) against *Escherichia coli* in water. The initial bacterial concentration was 10^5–10^6 CFU mL^{-1}. The error bars represent the standard deviation from the mean. The asterisk (*) indicates that the bacterial load was completely inactivated.

between the equivalent UV dosage and synergy value ($r = 0.88$, $p < 0.0001$). Accordingly, a 1.1-log average synergistic value was achieved within 75 min by single-UV + LTF combined treatment, and a 2.0-log average synergistic value was obtained within 60 min by double-UV + LTF combined treatment. These results indicated that a combination of high UV dosage and LTF induced a higher synergistic bactericidal effect against *E. coli* within a short exposure time.

The bactericidal activity of UV radiation is dependent on the physical light adsorption process. However, the adsorption of UV irradiation by water is limited by impurities, thus limiting its bactericidal activity (Tsolaki & Diamadopoulos 2010; Farrell *et al.* 2018). In this study, it could be speculated that the combined treatment improved contact between UV radiation and water, which facilitated bacterial cell penetration by UV radiation and improved UV bactericidal activity (Dang *et al.* 2020).

3.2. Bactericidal effect of ozone treatment and combined O_3 + LTF treatment against *E. coli* in water

The results of the present study showed that both O_3 treatment alone and combined O_3 + LTF treatment (at a flow rate of 65 L min^{-1}) exhibited considerable bactericidal activity against *E. coli* (Figure 3). Particularly, there was a substantial decrease in the *E. coli* count with increasing O_3 content. Additionally, the combined O_3 + LTF treatment exhibited significantly higher bactericidal activity against *E. coli* than the O_3 treatment alone (Figure 3(a) and 3(b)). Notably, the combination of LTF technology and different O_3 treatment ratios increased the O_3 concentration of the water from 0.1 to 0.6 mg L^{-1} with an increasing O_3 ratio (25–100%). In contrast, the O_3 concentration of the water was ~0.2 mg L^{-1} at 100% O_3 treatment in the absence of LTF technology, with the O_3 concentrations at lower O_3 supply rates (25–75%) below the detection limit (<0.1 mg L^{-1}). These results indicated that LTF technology considerably improved O_3 solubilization in water.

Furthermore, O_3 treatment alone exhibited considerable disinfection efficiency against *E. coli*, with 100% O_3 treatment inducing a 1.8-log decrease in the *E. coli* count, followed by 75% O_3 treatment (1.4-log decrease), 50% O_3 treatment (0.4-log decrease), and 25% O_3 treatment (0.3-log decrease) (Figure 3(a)). In the case of the combined O_3 + LTF treatment, there was a considerable increase in the disinfection efficiency against *E. coli* with increasing O_3 supply rates. Additionally, there was a positive correlation between the O_3 + LTF disinfection efficiency against *E. coli* and the O_3 concentration of the water ($r = 0.81$, $p < 0.0001$). Particularly, O_3 + LTF combined treatment exhibited higher bactericidal activity against *E. coli* than O_3 treatment alone at all O_3 supply rates, with 100% O_3 + LTF treatment inducing a 3.4-log decrease in *E. coli* count after 75 min, followed by 75% O_3 + LTF (3.2-log decrease), 50% O_3 + LTF (0.5-log decrease), and 25% O_3 + LTF (0.4-log decrease) (Figure 3(b)). Furthermore, the O_3 + LTF combined treatment exhibited synergistic effects against *E. coli* at 25, 50, 75, and 100% O_3 supply rates, with average synergy values of 0.1, 0.1, 1.8, and 1.5 log, respectively, within 75 min. Moreover, there was a significantly positive correlation between the dissolved O_3 concentrations and synergy values ($r = 0.84$, $p < 0.0001$), indicating that the synergistic effect of the combined O_3 + LTF treatment depended on the dissolved O_3 level of the water.

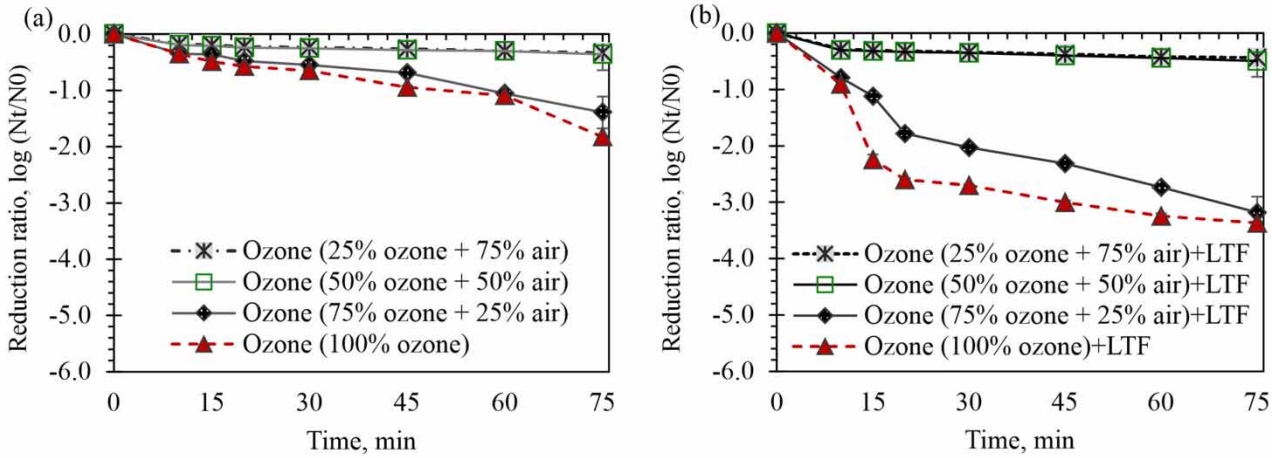


Figure 3 | *Escherichia coli* inactivation by (a) ozone alone and by (b) combined O_3 + LTF (at a flow rate of 65 L min^{-1}) treatment with different ozone supply rates (25% O_3 + 75% air, 50% O_3 + 50% air, 75% O_3 + 25% air, and 100% O_3). The initial bacterial concentration was 10^5–10^6 CFU mL^{-1}. Error bars represent the standard deviation from the mean.

The low disinfection efficacy of O_3 treatment alone confirmed that O_3 has poor solubility in water (Cho *et al.* 2003). In this study, LTF technology significantly increased the disinfection efficiency of O_3 in water. The use of LFFA allows the conversion of all liquids into LTFs and promotes water movement in the system, which increases the gas–liquid contact area by the interior interface (gas bubbles and liquid film) (Imai & Zhu 2011; Imai & Dang 2017). It is assumed that LTF technology can considerably improve O_3 solubility in water because of the larger surface area and longer residence time of the gas bubbles in water (Imai & Zhu 2011; Nguyen *et al.* 2018), which enhance O_3 diffusion into bacterial cells. O_3 is an extremely powerful disinfectant, mainly because of its ability to oxidize substances in bacterial cell membranes. When O_3 penetrates the phospholipid layer, it can damage the cell wall structure (Ding *et al.* 2019) and destroy the membrane surface (Wu *et al.* 2018; Ding *et al.* 2019).

3.3. Bactericidal activity of combined UV + ozone + LTF treatments against *E. coli* in water

The disinfection efficacy of the combined single-UV + O_3 + LTF (Figure 4(a)) and double-UV + O_3 + LTF (Figure 4(b)) treatments against *E. coli* was investigated using four O_3 supply rates of 25% O_3 + 75% air, 50% O_3 + 50% air, 75% O_3 + 25% air, and 100% O_3, and LTF technology (water flow rate of 65 L min^{-1}) for 75 min. The bacterial concentration of the samples before treatment ranged from 10^5 to 10^6 CFU mL^{-1}. The result of the present study showed that there was a considerable increase in the disinfection efficiency of the combined treatments with increasing UV dosages and O_3 rates (Figure 4). Additionally, the disinfection efficacy of UV + O_3 + LTF combined treatment against the pathogen was higher than those of individual treatments, and positive synergistic values were observed for the combined treatments.

Furthermore, the bactericidal activity of the combined treatments against *E. coli* was significantly influenced by both the UV dosage and O_3 supply rate (Figure 4(a) and 4(b)). Particularly, single-UV + 100% O_3 + LTF combined treatment caused a 5.1-log decrease in the *E. coli* count, followed by single-UV + 75% O_3 + LTF (4.4-log decrease), single-UV + 50% O_3 + LTF (4.1-log decrease), and single-UV + 25% O_3 + LTF, with the lowest efficacy (3.9-log decrease). The results showed that higher O_3 ratios enhanced the bactericidal efficacy of the combined UV + O_3 + LTF treatment, confirming the findings reported in the previous section. A similar relationship was observed between the O_3 supply rate and disinfection efficiency of the combined double-UV + O_3 + LTF treatment, although the highest inactivation efficiency and synergistic benefits were observed at a UV dosage of 41.7 mJ cm^{-2} (double-UV lamps, Figure 4(b)). The treatment period required for

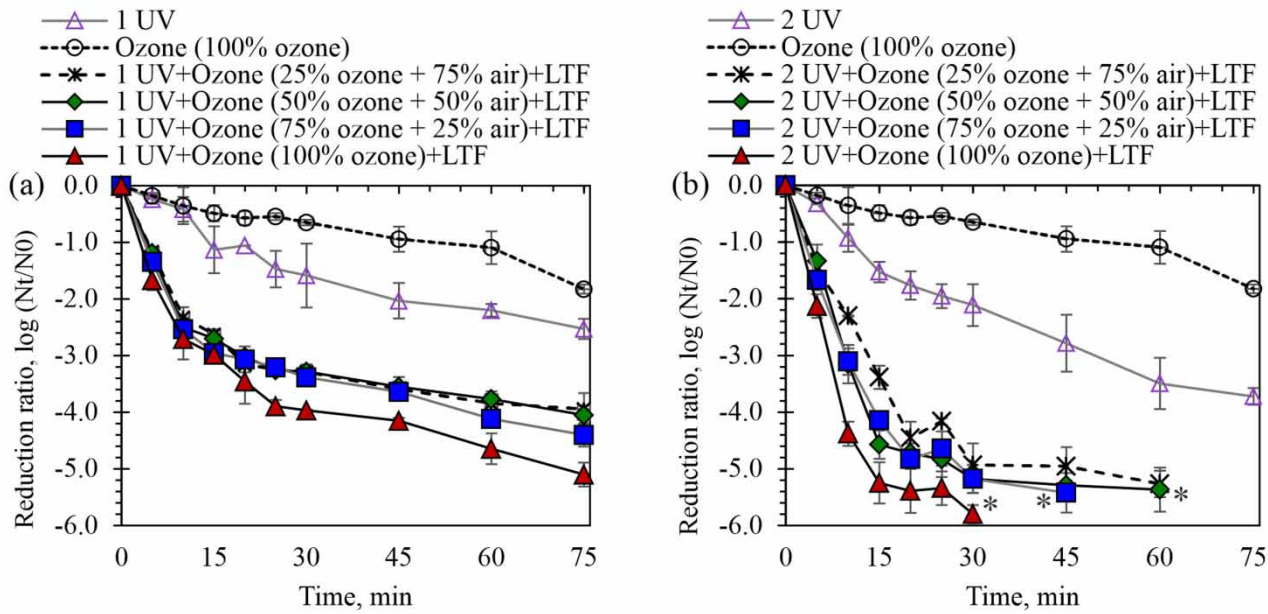


Figure 4 | *Escherichia coli* inactivation by (a) the combined single-UV + O_3 + LTF and (b) the combined double-UV + O_3 + LTF under different ozone supply ratios of 25% O_3 + 75% air, 50% O_3 + 50% air, 75% O_3 + 25% air, and 100% O_3. The initial bacterial concentration was 10^5–10^6 CFU mL^{-1}. Error bars represent the standard deviation from the mean. The asterisk (*) indicates that the bacterial load was completely inactivated.

the complete inactivation of *E. coli* decreased with increasing O_3 percentage (60 min at 25% O_3, 60 min at 50% O_3, 45 min at 75% O_3, and 30 min at 100% O_3).

Notably, UV + O_3 + LTF combined treatments exhibited synergistic benefits against the pathogen at all UV and O_3 dosages (Supplementary material, Table S1). Particularly, the synergy values of the single-UV + O_3 + LTF combined treatment under 25, 50, 75, and 100% O_3 conditions were 1.1, 1.2, 0.5, and 0.7 log within 75-min treatment, whereas those of double-UV + O_3 + LTF combined treatment were 1.5 (25% O_3) and 1.6 (50% O_3) log after 60 min, 2.0 log (75% O_3) after 45 min, and 3.0 log (100% O_3) within 30 min of treatment. These data suggest that higher UV dosages and O_3 supply ratios with shorter exposure times or lower UV dosages and O_3 supply ratios with longer exposure times can be applied to inhibit bacteria.

Furthermore, the O_3 concentrations of water samples treated using the UV + O_3 + LTF combined treatment and O_3 ratios of 25, 50, 75, and 100% reached 0.1, 0.2, 0.3, and 0.6 mg L^{-1}, respectively, within 5–15 min of treatment. However, the concentrations dropped below the detection limit (<0.1 mg L^{-1}) after 30 min of treatment. Further experimentation after the treatment periods revealed no regrowth potential for *E. coli* in the dark after exposure to the double-UV + O_3 + LTF combined treatment.

Jung *et al.* (2008) reported that combined O_3 (2 mg L^{-1}) and UV radiation (14 mJ cm^{-2}) treatment exhibited synergistic bactericidal effects against *Bacillus subtilis* spores. Additionally, Blatchley *et al.* (2012) reported that combined UV_{254} (12.4 mJ cm^{-2}) and O_3 (2–3 mg L^{-1}) treatment did not exhibit any synergistic effect against *E. coli* in wastewater; however, combined double-UV + LTF disinfection completely inactivated more than 5.0-log *E. coli* and exhibited 2.0-log synergy after 60 min. In addition, combined double-UV (41.7 mJ cm^{-2}) + O_3 (100% O_3, 0.6 mg L^{-1}) + LTF (65 L min^{-1}) treatment reduced the treatment period to 30 min, with average synergy value of 3.0 log. These results confirm the bactericidal efficacy of the combined UV + O_3 + LTF disinfection method, indicating its potential future application for water treatment.

Although the disinfection mechanisms of the combined UV + O_3 + LTF treatment are yet to be fully elucidated, some hypotheses could be drawn. The disinfection efficacy of dissolved O_3 depends on its rapid penetration of bacterial cell membranes, where it oxidizes material, resulting in cell rupture and death (Wu *et al.* 2018; Ding *et al.* 2019). Additionally, UV radiation at 254 nm can induce the formation of hydroxyl radicals ($^{\cdot}OH$, $E^0 = 2.80$ V/NHE) from photolytic O_3 decomposition, which may further contribute to the increase in inactivation rates (Chin & Bérubé 2005; Jung *et al.* 2008). The findings of this study revealed that the combined disinfection methods (UV + O_3 + LTF) were effective against the pathogen, which could largely be attributed to the improved gas–water interaction efficiency as well as the water circulation caused by the LFFA's action, thus improving cell penetration by UV radiation and O_3 efficacy against the bacteria. The better results obtained from the combined UV + O_3 + LTF treatment against *E. coli* could be attributed to the better diffusion of UV light into water (Dang *et al.* 2020), better dissolution of O_3 in water, and generation of hydroxyl free radicals (Chin & Bérubé 2005; Jung *et al.* 2008; Takahashi *et al.* 2021).

Moreover, increasing the contact between UV and *E. coli* may induce radiation damage to nucleic acids in the bacterial cells, leading to cell replication dysfunction, mutagenesis, irreversible damage, and cell death (Xu *et al.* 2018). UV radiation can cause lipid peroxidation and breakdown of protein-like materials, as well as genetic damage, at high doses, thereby altering the bacterial cell membrane (Xu *et al.* 2018). Unlike UV disinfection, the bactericidal activity of O_3 is related to its oxidation of the constituents of the cell wall (such as proteins and amino acids), damage to cellular structures, release of intracellular substances (Ding *et al.* 2019), and destruction of the membrane surface (Wu *et al.* 2018; Ding *et al.* 2019). Overall, it can be concluded that the concomitant effects of UV dosage and O_3 concentration increased the sensitivity of the bacterial cells to the combined UV + O_3 + LTF treatment.

4. CONCLUSIONS

The findings of this study illustrated that LFFA significantly improved the disinfection efficiency of UV and O_3 treatments individually and as a combined treatment. The bactericidal efficacy of the treatments and their synergistic effects were in the order of combined double-UV + O_3 (100% O_3) + LTF treatment > combined double-UV + LTF treatment > combined O_3 (100% O_3) + LTF treatment > double-UV alone > O_3 (100% O_3) alone. Additionally, the combined UV + O_3 + LTF treatment exhibited considerably higher bactericidal efficacy than either UV or O_3 treatment alone. The simultaneous application of UV (41.7 mJ cm^{-2}), O_3 (100% O_3), and LTF (65 L min^{-1}) reduced the bacterial load of *E. coli* by approximately 5.8 log within 30 min, while the sum of the log reductions due to the individual treatments was approximately 2.8 log. The synergistic effect of the combined treatment was attributed to the simultaneous enhancement of three main factors: UV radiation transmittance, aqueous ozone solubility, and hydroxyl radical formation.

In summary, the findings of this study highlight the bactericidal activity and synergistic effect of combined UV + O_3 + LTF treatment and indicate that the method possesses a potential for use in future applications for water disinfection. However, further studies are necessary to elucidate the mechanism of DBP formation during disinfection and the possibility of applying this treatment to other kinds of microorganisms.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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