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Kinetics of inactivation and photoreactivation of *Escherichia coli* using ultrasound-enhanced UV-C light-emitting diodes disinfection

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ABSTRACT

Ultraviolet (UV) disinfection is highly recommended owing to its high disinfection efficiency and disinfection by-products free, and UV Light-Emitting Diodes (UV LEDs) is increasingly becoming an alternative of mercury UV lamps for water disinfection owing to its long lifetime, low input power, and absence of problems on disposal. However, renovation of existing UV lamps faces the challenges for UV disinfection associated with disinfection efficiency and photoreactivation, and modified UV disinfection process is required for practical application. In this study, mathematical rule of disinfection and photoreactivation in a US enhanced UV disinfection system was investigated. UV LED with peak emission at 254 nm (UV-C LED) was selected as representative for UV lamps, and a low frequency US was used as pretreatment followed by UV disinfection. The disinfection efficiency of *Escherichia coli* in deionized water (DI), DI water with kaoline suspension (DIK), and secondary effluent (SE) of municipal wastewater treatment plant were analyzed. Moreover, photoreactivation of *E. coli* in DIK water within 6 h after disinfection was conducted. The experimental results showed that the disinfection efficiencies had good fit with Chick-Watson first-order linear model, and US pretreatment increased the inactivation rate constant for *E. coli*, which increased from 0.1605 to 0.1887 in the DIK water. Therefore, US pretreatment with UV disinfection have potential to shorten the retention time and reduce the reactor volume. Moreover, the number of photoreactivated *E. coli* in effluent was reduced under UV-C LED disinfection with US pretreatment compared with that under UV-C LED disinfection alone. The order of maximum percentage of photo-reactivated *E. coli* was as follows: UV-C LED disinfection alone at 30 mJ/cm^2 > UV-C LED disinfection at 25 mJ/cm^2 with US pretreatment > UV-C LED disinfection at 30 mJ/cm^2 with US pretreatment. The survival ratio versus photoreactivation time showed a good fit to second-order logistic model. US pretreatment in UV-C LED disinfection could improve disinfection efficiency, reducing photoreactivation in the effluent as well, which offers a promising practical application technology.

1. Introduction

Ultraviolet (UV) disinfection has been one of the most attractive methods for water and wastewater disinfection owing to its broad-spectrum efficacy against pathogens and non-formation of disinfection by-products [1]; thus, the application of UV disinfection rapidly increased worldwide. In 1982, 14 wastewater treatment plants (WWTPs) in the United States employed UV disinfection systems, which were funded by the Environmental Protection Agency [2]. Thereafter, more than 30 countries or regions, including North America, European Union, Asia, and Austria, have subsequently established UV disinfection facilities for water and wastewater treatments, resulting in more than 3000 WWTPs. In China, 50% of municipal WWTPs used UV disinfection in 2002 [3]. Nowadays, mercury UV lamp is dominantly employed for UV disinfection, especially for low pressure mercury lamp. However, UV disinfection faces challenges such as relatively high operation cost compared with chemical disinfection, fouling and decay of UV lamps, as well as problems related to mercury disposal. Therefore, attention has been devoted on the development of a new type of UV light. One of the most potential alternatives is UV light-emitting diodes (UV LED). UV LED is a semiconductor p–n junction device that can produce electroluminescence and emit a narrow spectrum of light [4,5]. Compared with mercury UV lamps, UV LED offers many advantages such as extreme long life of 100,000 h and low energy consumption given that UV LED requires low voltages and low power. UV LED is mercury-free and thus its disposal is not a problem [6–8]. Moreover, UV LED efficiently transforms energy into light, and its electrical-to-germicidal efficiency

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can reach up to 75% in some cases. Therefore, UV LED is a promising technology for a more efficient disinfection.

DNA mainly absorbs UV light with wavelengths between 200 and 300 nm, but the absorbed peak wavelength is dependent on the different target organisms. Low pressure mercury lamps emit nearly monochromatic UV light at wavelength of 254 nm, while UV LED could be manufactured at different peak emission wavelength from 250 nm to 340 nm for microbial disinfection, making it possible use intended combined wavelength. However, UV-Hg lamps and UV LED share the same disinfection mechanism, i.e. UV irradiation can damage DNA, and the disinfection process is easily affected by water quality such as turbidity, size and concentrations of suspended solids, and water color [9,10]. UV irradiation could reach the free-swimming bacteria easily, but the bacteria protected by suspended particles is still remained in the disinfected effluent. Under such a circumstances, UV irradiation is partly absorbed by the particles, and which results in low disinfection efficiency [11]. Therefore, breaking down the particles into small pieces to release more free-swimming bacteria is essential for high disinfection efficiency.

Ultrasound (US) has been introduced to improve the efficiency of UV disinfection and the enhanced effects have been reported in previous studies [12,13]. It was demonstrated that low frequency US could break down bacteria flocs by mechanical shear force, and changing the particle size distribution consequently [14]. When US was operated at an input power density of 30 W/L for 30 s, the particles larger than 50 μm could be reduced from 63% to 5%, so that the disinfection effect increased [15]. In our previous investigation by a baffled US/UV disinfection reactor at a pilot scale, it was also found that US pretreatment or simultaneous US/UV disinfection could improve the disinfection efficiency with 0.4 and 0.5 log compared with UV disinfection alone without increasing the specific energy consumption [16]. Moreover, multiple functions of US in the US pretreatment or simultaneous treatment with other disinfectants have been figured out, including de-agglomerating, cell damage, as well as sonolysis, among them de-agglomerating is a dominate effect [17]. Till now, it is almost clear to all that US could improve the disinfection efficiency, but the photoreactivation of inactivated bacteria after UV disinfection is a great concern for decision makers. In a promoted modelling of photoreactivation, it was supposed that the low survival ratio after disinfection could lead to a low photoreactivation ratio [18]. Since US enhanced the disinfection efficiency, the survival ratio decreased, the photoreactivation ratio seemed to decrease accordingly. However, no literature could be found about this.

As aforementioned, the kinetics of inactivation and photoreactivation of *Escherichia coli* in ultrasound-enhanced disinfection system have been investigated. A Pearl Beam of UV LED at 254 nm wavelength was employed for UV disinfection, and US was introduced as pretreatment for disinfection enhancement. The objective of this research is to provide the theoretical and experimental basis for US enhanced UV disinfection technology.

2. Material and methods

2.1. Water samples

This study used deionized (DI) water, DI water with kaoline suspension (DIK), and secondary effluent (SE) of municipal WTTP as test media.

2.1.1. Bacterial suspension

Escherichia coli (ATCC 15597) was used as indicator microorganism. For each experiment, 20 μL of *E. coli* (ATCC 15597) strain was added into 50 mL of LB broth, placed in an incubator shaker at 37 °C, and shaken at 130 r/min for 12 h. The *E. coli* suspension was subsequently centrifuged at 4000 r/min for 10 min. The spores were washed in phosphate buffered saline twice and then resuspended in 30 ml of sterilized DI water to prepare an *E. coli* concentration of approximately 10^9 CFU/ml. Before performing the irradiation experiments, the *E. coli* was diluted with sterilized DI water to achieve an initial concentration of 10^8 CFU/ml. Similarly, *E. coli* was diluted with sterilized DI water containing 20 mg/L kaoline.

2.1.2. Secondary effluents of municipal WTTP

Water samples were collected from the outlet of a secondary treatment unit in a municipal WWTP of Fangshan District, Beijing, which mainly utilizes BIOLAK for treatment.

Tests were performed using 30 ml of stationary samples. Absorbance was measured on HACH DR6000 UV/Vis Spectrophotometer (HACH, USA), and turbidities were measured using HACH 2100P ISO turbidimeter.

2.2. Disinfection process

The whole disinfection process is presented in Fig. 1, and all the experiments were done in batch reactor. Water samples were treated by sonication in the US reactor, and then moved to the UV irradiation for further treatment.

A low-frequency US (33 kHz) with adjustable power from 0 W to 200 W (Hainertec Ultrasonic Technology Co., Ltd., Suzhou, Jiangsu Province, China) was used for US pretreatment. Water sample (2 L) was added into an ultrasonic reactor and then irradiated with US for 40 s with an applied input power density of 66 W/L, which is equal to a specific energy consumption of 2.64 kJ/L [17]. The real US input power was measured using a power meter (LCDG-ZJ1-62010). Once US sonication was completed, 30 ml of the sonicated water samples were transferred into a Petri dish.

The main disinfection process was performed using a collimating beam device (PearlBeam: PB-S-255-L, AquiSense Technologies, North Carolina, USA). Table 1 shows the technical specification of the device.

The UV-C LED collimated beam was fixed at 20 mm above the surface of a sample (30 ml) contained in a sterilized Petri dish (90 mm in inner diameter). Magnetic stirrer was used to sufficiently mix the suspension throughout its exposure to UV. The irradiation time was controlled to 0, 150, 300, 450, 600, 750, and 900 s to achieve UV doses of 0, 5, 10, 15, 20, 25, and 30 mJ/cm^2, respectively. All experiments were conducted at room temperature. After disinfection, the prepared solutions were sampled for microbial analysis.

2.3. Photoreactivation process

A UVA lamp (8 W, 365 nm) was used as light source for photoreactivation. UV-irradiated water samples were circled around the lamp to ensure that they receive the same irradiation intensity during reactivation; samples were periodically obtained at a given time interval for a total of 6 h. The experiment was conducted at room temperature.

2.4. Enumeration

After irradiation by the UV-C LED system, 0.1 ml of *E. coli* suspension was properly diluted and plated on nutrient agar plates. The plates were incubated for 24 h before enumeration at 37 °C.

E. coli concentrations in SE before and after disinfection were detected by membrane filter method according to the U.S. EPA Method 1604 [19]. Appropriate amounts of water samples were filtered through a 0.45 μm filter (50 mm, polyvinylidene fluoride,

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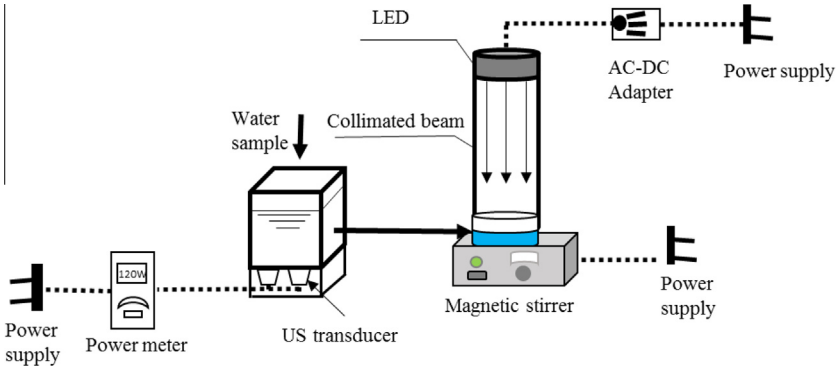

Fig. 1. Schematic of the batch reactor.

Table 1
Technical specifications of the collimating beam device.

	255 nm
Peak Wavelength (nm)	254.2
Full width half maximum (nm)	11.3
Radiant flux (mW)	5.2
Average intensity 3 mm from collimator end (μW/cm^2)	35

China), and then the filter was placed in MI broth incubated at 35 °C for up to 24 h. The filter was subsequently transferred into MUG broth incubated at 35 °C for another 4 h, and the colonies appeared blue under fluorescent light. Each sample was plated in triplicate, and plates yielding 0–100 colonies were counted; data were presented as mean values.

2.5. Statistical analysis

Disinfection efficiencies were experimentally evaluated by logarithmic reduction; inactivation values were expressed as log (N_0/N), where N_0 and N represent the number of microorganisms in the samples before and after disinfection, respectively.

For reactivation experiments, the percentage of surviving bacteria and the percentage of photoreactivation was determined as follows [20,21]:

$$\text{Percentage of survival after disinfection } (\%) = \frac{N_d}{N_0} \times 100\% \quad (1)$$

$$\text{Percentage of survival after photoreactivation } (\%) = \frac{N_p}{N_0} \times 100\% \quad (2)$$

$$\text{Percentage of photoreactivation } (\%) = \frac{N_p - N_d}{N_0 - N_d} \times 100\% \quad (3)$$

where N_p represents the number of bacteria in a reactivated sample (CFU/L), N_d represents the number of bacteria immediately after disinfection of effluent, and N_0 represents the initial bacterial concentration before disinfection (CFU/L).

2.6. Modelling

2.6.1. Inactivation kinetics

Linear relationship between log inactivation and the applied UV dose is always used to describe a disinfection model, and the formulation of Chick-Watson first-order linear model is as follows [22]:

$$\log(N_0/N_d) = k^* Dose \quad (4)$$

where N_0 and N_d represent the number of bacteria before and after disinfection, respectively.

Moreover, a model between log inactivation and the applied UV dose can be expressed as follows [7]:

$$DR = k^* Dose - b \quad (5)$$

where DR is the decimal reduction factor (=log N_0/N_d), k is the inactivation rate constant, and b is the offset value with a negative value, which crosses the fluence axis at the fluence where logloner relationship starts.

2.6.2. Photoreactivation kinetic

Kashimada et al. proposed an asymptotic model demonstrating that photoreactivation after UV disinfection follows a first-order reaction (Eq. (6)). Furthermore, NebotSanz et al. [18] proposed a modified photoreactivation model based on saturation-type first-order reaction, and the kinetics can be expressed as follows:

$$S = (S_m - S_0)(1 - e^{-k_1 t}) + S_0 \quad (6)$$

$$S = \frac{S_m}{1 + \left[\frac{S_m}{S_0} - 1\right] \exp^{(-k_2 \cdot S_m \cdot t)}} \quad (7)$$

where S_0 is the survival immediately after UV disinfection (N/N_0), S_m is the maximum limit of the survival of microorganism resulting from reactivation, k_1 is the first-order reactivation rate constant, k_2 is the rate at which that value is reached, and t is the reactivation time.

3. Results and discussion

3.1. Disinfection efficiency

Fig. 2 shows the disinfection efficiency of UV-C LED in water samples at 254 nm with or without US pretreatment. The inactivation results verified the reproducibility of disinfection ability in water with different qualities. The highest inactivation rate was achieved in DI water when UV-C LED was solely employed for disinfection; this result is attributed to the differences in water medium, as shown in Table 2. Turbidity and absorbance of DIK water at 254 nm were higher than those of DI water; inorganic particles in DIK water could protect the bacteria from irradiation and prevent them from absorbing UV, resulting in the escape of some bacteria from irradiation; thus, disinfection efficiency decreased. In SE, the density of *E. coli* in water was considerably low (approximately 10^4 CFU/L) and thus the disinfection efficiency decreased accordingly, although the turbidity and absorbance were relatively low. All disinfection efficiencies under US pretreatment increased compared with those under disinfection with UV-C LED alone. An

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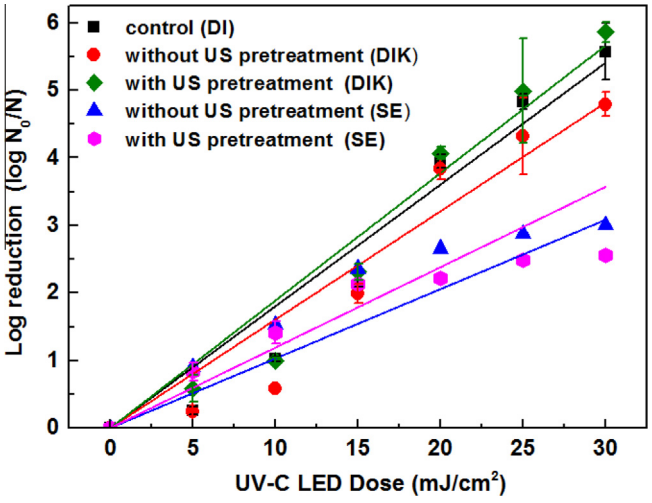

Fig. 2. Inactivation response of *Escherichia coli* in different water samples to UV disinfection.

Table 2
Turbidity and absorbance of different test media before and after US treatment.

Test medium	Turbidity (NTU)	Absorbance at 254 nm
DIK	77.4	0.816
DI	31.5	0.609
SE	1.91	0.151
DIK*	74.4	0.829
DI*	33.2	0.610
SE*	2.68	0.118

Note: the remark "*" meant for the water samples after US pretreatment.

increase of 0.24, 0.41, 0.32, 0.25, 0.67, and 1.07 log for each dosage was observed in DIK water, whereas an increase of 0.07, 0.12, 0.23, 0.44, 0.39, and 0.55 log was observed for each dosage in SE water, indicating the enhancement effect of US in the combined disinfection technology. Different tendencies of disinfection efficiency were observed in SE and DIK water (Fig. 2). For SE, the disinfection efficiency increased gradually with increasing UV dose, and tailing effect was observed given that *E. coli* concentration was low in the initial disinfection influent, indicating that most of the *E. coli* were inactivated at 20 mJ/cm^2 dose. However, for DIK water, the disinfection efficiency was very low before 15 mJ/cm^2 and then suddenly increased at 20 mJ/cm^2 up to 30 mJ/cm^2. This is mainly due to water quality, as it can be found in Table 2 that turbidity and light absorbance were high in DIK water, UV irradiation was partly absorbed by water, and reducing the dose reach to bacteria. With longer irradiation time, accumulated UV dose could exert lethal effect on the bacteria, thus performed a sudden increase in log reduction at the dosage of 20 mJ/cm^2.

For a more insightful analysis of the disinfection efficiency, two regressions using all data points between 0 mJ/cm^2 and 30 mJ/cm^2 were performed according to Eqs. (4) and (5) mentioned in Section 2.5. Tables 3 and 4 show the values of the estimated kinetic parameters. High goodness of fit of the Chick-Watson linear model was observed, and the following discussion is based on this mathematical model.

Under irradiation with UV-C LED alone, the sequence of the inactivation rate constant k was as follows: DI water > DIK water > SE water. By contrast, the sequence when US pretreatment was employed followed an order as follows: DIK water > DI water > SE water. The inactivation rate constant is higher under irradiation with US pretreatment than that under UV-C LED irradiation alone, and the disinfection effect in SE water is theoretically better than the one in DI and DIK, but the maximum inactivation rate constant k (0.1887) was achieved in US-pretreated DIK water, this is due to the higher *E. coli* concentration. As aforementioned, the bacteria concentration in SE is much lower than the bacteria in DI and DIK water, which were about 10^4 CFU/L and 10^8 CFU/mL, respectively, thus the inactivation rate of SE water is lower. We previously demonstrated that US-enhanced disinfection was mainly dependent on de-agglomerating effect caused by mechanical shearing effect of US, which released more particle-associated bacteria to UV irradiation. Besides, the release of hydroxide radical (·OH), mechanical sharing force and heat effect produced by US through cavitation performed joint results on cell walls at the same time, then together with de-agglomerating improving the UV disinfection process accordingly [16]. Table 2 shows that US pretreatment exerted no influence on water turbidity and absorbance at 254 nm, further demonstrating the de-agglomerating effect. When kaoline was distributed in *E. coli* suspension, some bacteria would be adsorbed or wrapped in kaoline-formatted particles, shading or scattering UV irradiation from the bacteria. Under US pretreatment, the particle-associated bacteria were again exposed to UV irradiation; this phenomenon, together with the sub-lethal damage, increases the disinfection efficiency. As a result, the inactivation constant increased obviously and nearly equaled that in DI water inactivation, indicating that US eliminated the negative effect associated with inorganic particles in water. The initial *E. coli* concentration was considerably low in SE, leading to a low

Table 3
Kinetic parameters of Chick-Watson linear model applied in inactivation experiments.

Water samples	Disinfection process	k	SE	R^2
DI	Control	0.1802	0.0109	0.974
DIK	Without US pre-treatment	0.1605	0.0121	0.961
DIK	With US pre-treatment	0.1887	0.0101	0.980
SE	Without US pre-treatment	0.1027	0.0081	0.957
SE	With US pre-treatment	0.1189	0.0083	0.966

Table 4
Kinetic parameters of Shoulder model applied in inactivation experiments.

Water samples	Disinfection process	k	SE	b	R^2
DI	Control	0.2275	0.0153	−1.0352	0.977
DIK	Without US pre-treatment	0.2046	0.0235	−0.956	0.937
DIK	With US pre-treatment	0.2294	0.0170	−0.88267	0.972
SE	Without US pre-treatment	0.0684	0.0122	0.742	0.8573
SE	With US pre-treatment	0.0851	0.0135	0.7333	0.8855

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experimental inactivation rate constant. However, US pretreatment increased the inactivation rate accordingly from 0.1027 to 0.1189, accounting for about 15% increase.

Nevertheless, the enhanced inactivation rate constant was lower than the reported value both for UV lamps and UV LEDs. The k values achieved for *E. coli* 11229 under UV-C LED and mercury UV lamps were 0.300 and 0.506, respectively. The different results obtained in this study demonstrated that under similar total UV dose, high flux and short exposure time resulted in a higher log inactivation than that under low flux and long exposure time [23]. *E. coli* 15597 is the most resistant *E. coli* strain to UV [24] and thus caused the low inactivation constant in UV-C LED tests.

3.2. Photoreactivation

Fig. 3 shows the potential photoreactivation of *E. coli* in the water samples. DIK water was used in this investigation; disinfection with or without US pretreatment were conducted, and bacterial counts were monitored for 6 h after UV irradiation. *E. coli* concentrations in samples were measured at 0, 0.5, 1, 2, 3, 4, 5, and 6 h, where t = 0 means the effluent after UV disinfection at that experimental condition. Dark repair and regrowth of bacteria play only a small part in reactivation [25]; thus, the amount of *E. coli* in the samples after irradiation indicated the degree of photoreactivation.

Fig. 3 shows that the number of *E. coli* immediately increased following exposure to photoreactivating light at 30 mJ/cm^2 during disinfection with UV alone. The number of active *E. coli* cells per milliliter of water sample increased to nearly 10^7 CFU after 5 h, which was the highest number of *E. coli* that survived under US pretreatment followed by UV disinfection at 30 and 25 mJ/cm^2. Moreover, all of the effluents reached the maximum number of *E. coli* after 5 h, and the maximum number was ranked as follows: UV disinfection at 30 mJ/cm^2 > UV disinfection at 25 mJ/cm^2 with US pretreatment > UV disinfection at 30 mJ/cm^2 with US pretreatment, and the photoreactivation ratios were 9.9%, 7.1% and 2.6% accordingly. Fig. 4 shows the concentration of *E. coli* in disinfected effluent and the maximum photoreactivation concentration. With US pretreatment, the disinfection effluents improved, and the photoreactivation could be controlled. Furthermore, under disinfection with the same UV dosage (30 mJ/cm^2) with US pretreatment, the disinfection efficiency increased from 3.70 log to 4.92 log without causing any increase in *E. coli* density during photoreactivation. The maximum reactive number of *E. coli* was nearly 10^6 CFU/ml when US pretreatment was employed. Therefore, US pretreatment could enhance UV disinfection efficiency and reduce the degree of photoreactivation.

Fig. 5 shows the survival ratio versus exposure time to photoreactivation in the three disinfection processes as revealed by the

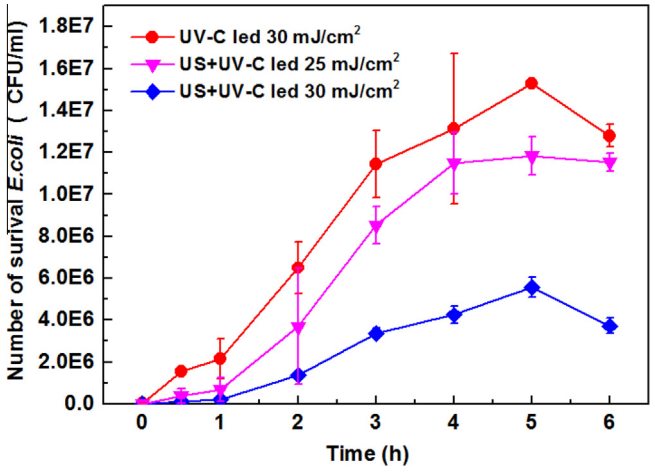

Fig. 3. Photoreactived concentration of *Escherichia coli* after disinfection under UV-C LED alone at 30 mJ/cm^2, US pretreatment prior to UV-C LED disinfection at 25 mJ/cm^2, and US pretreatment prior to UV-C LED disinfection at 30 mJ/cm^2.

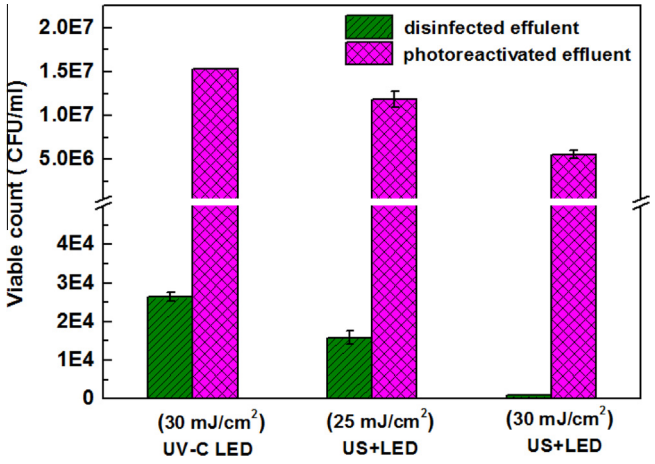

Fig. 4. Viable counts of *Escherichia coli* resulting from photoreactivation under 365 nm UVA light after UV-C LED disinfection alone at 30 mJ/cm^2, after UV-C LED disinfection at 25 mJ/cm^2 with US pretreatment, and after UV-C LED disinfection at 30 mJ/cm^2 with US pretreatment.

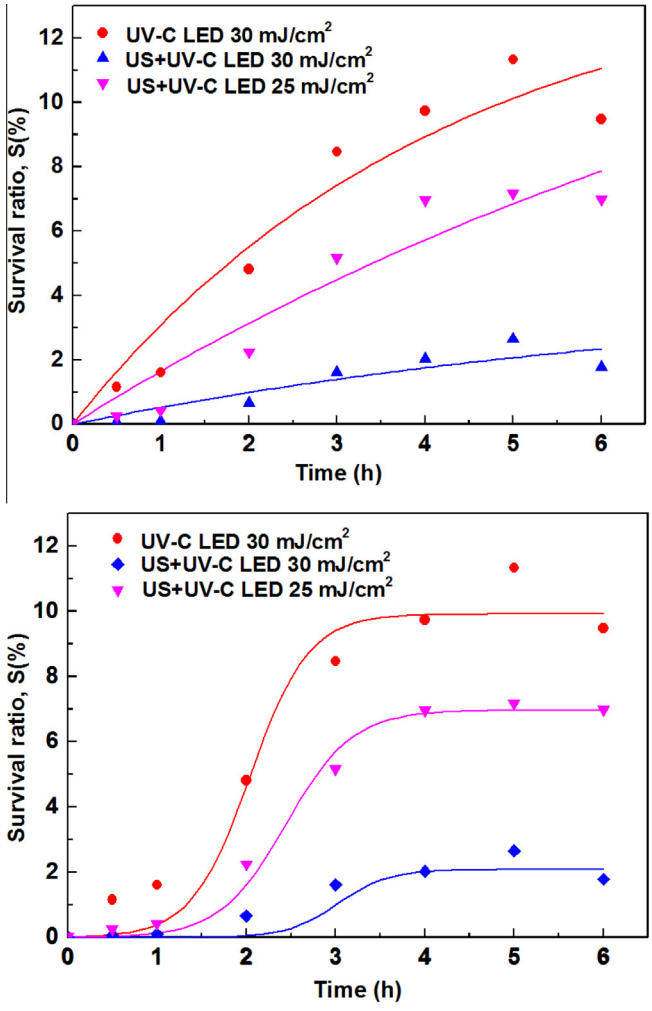

Fig. 5. Survival ratio versus exposure time to photoreactivation in different disinfection processes as revealed by logistic regression model.

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Table 5
Kinetic parameters of the first-order model applied in photo-reactivation experiments.

Disinfection process	Sm (observed)	Sm (predicated)	SE	K_1	SE	R^2
30 mJ/cm^2	11.3333	14.55851	4.31773	0.23781	0.12199	0.92828
US + 25 mJ/cm^2	7.17170	17.89068	18.59691	0.09647	0.12646	0.91271
US + 30 mJ/cm^2	2.65080	4.32848	4.71865	0.13022	0.1936	0.82472

Table 6
Kinetic parameters of the logistic model applied in photo-reactivation experiments.

Disinfection process	Sm (observed)	Sm (predicated)	SE	k_2	SE	R^2
30 mJ/cm^2	11.3333	9.91926	0.51294	0.30745	0.0282	0.95244
US + 25 mJ/cm^2	7.17170	6.97302	0.21787	0.38642	0.02189	0.98673
US + 30 mJ/cm^2	2.65080	2.08366	0.16524	1.69649	0.2473	0.91463

two logistic regression models (Eqs. (6) and (7)) mentioned in Section 2.5. Tables 5 and 6 show the values of the estimated kinetic parameters. The second-order logistic model showed a good fit to the experimental data, and the achieved maximum photoreactivation percentage is close to the value predicted by the regression model. The highest survival percentage after photoreactivation was reduced by US pretreatment to 7.1% and 2.6%, which are approximately 4% and 9% reduction.

To our knowledge, disinfection with UV-C alone damages DNA, although these damages are reparable in certain circumstances, especially during photoreactivation. One way to reduce the risk of photoreactivation in disinfected effluents is wavelength coupling to achieve both DNA damage caused by UVC (200–280 nm) and oxidative damage caused by UVA (315–400 nm); the synergistic effect of wavelength coupling in inactivation of mesophilic bacteria was observed previously [24]. However, few experiments have investigated the degree of photoreactivation under UV disinfection with US pretreatment. This study found that the number of reactivated *E. coli* during disinfection with US pretreatment was significantly lower than that under disinfection with UV alone, and even significantly lower than the reported levels of photoreactivation after UV disinfection alone [26,27]. Increase in UV dose reduces the concentration of photoreactivated *E. coli* resulting from irreversible damage caused by increased UV dose irradiation [28]. However, in this study, photoreactivation of *E. coli* after US pretreatment followed by UV disinfection at 25 mJ/cm^2 showed a lower "maximum" photoreaction level than that after UV disinfection at 30 mJ/cm^2 although the applied UV dosage decreased. Hence, US suppressed photoreactivation. The possible explanation for the effect of US pretreatment in reducing the photoreactivation could be in two ways. Firstly, as it can be seen in Eq. (7), the survival ratio is associated with the survival numbers after UV disinfection, in other words, it could be said that the lower concentration in the disinfection effluent, the lower photoreactivation ratio. It can be concluded that the US pretreatment enhanced the disinfection efficiency, and resulting in a low *E. coli* concentration in the effluent, therefore, the photoreactivation reduced. Secondly, it is hypothesized that the radicals ·OH generated during sonication create some sub-lethal damage effect on the cell walls and enzymes, changing the permeability of cell walls or blocking the enzymes' synthesis [29,30]. However, the exactly effect of US in the photoreactivation process is required for further verification.

4. Conclusions

This work performed lab-scale *E. coli* disinfection under UV-C LED alone emitted at 254 nm and under UV-C LED with US pretreatment. Different test media were used, and both disinfection and photoreactivation were investigated. The disinfection ability of UV-C LED was enhanced by US pretreatment. Inactivation of *E. coli* by UV-C LED and by US combined with UV-C LED fitted well into the Chick-Watson linear model, and the inactivation rate constant increased during UV disinfection with US pretreatment. US improved the disinfection in terms of increased disinfection efficiency and reduced photo-reactivation. The maximum photoreactivation of *E. coli* in disinfected effluent was observed after 5 h in all tested disinfection conditions, and the survival ratio versus photoreactivation time showed a good fit to second-order logistic model. Moreover, the maximum survival ratio decreased from approximately 11% to 2% under UV disinfection with US pretreatment. US exerted key physical and chemical effects in the combined disinfection process. The chemical effects are hypothesized to influence the repair enzymes, thereby improving the disinfection efficiency, although this phenomenon requires further research.

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