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RESEARCH ARTICLE

Bacterial inactivation, DNA damage, and faster ATP degradation induced by ultraviolet disinfection

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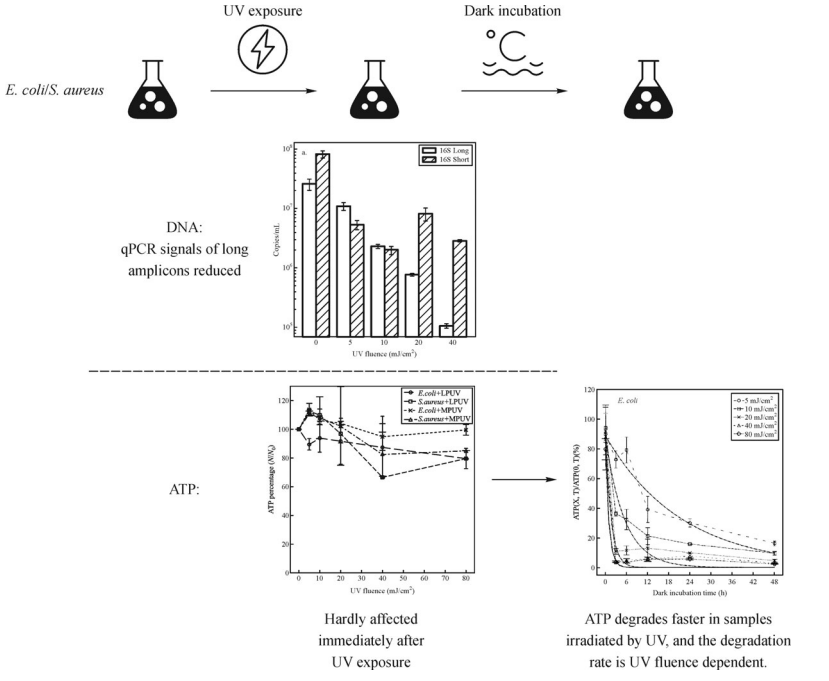
HIGHLIGHTS

- Long amplicon is more effective to test DNA damage induced by UV.
- ATP in bacteria does not degrade instantly but does eventually after UV exposure.
- After medium pressure UV exposure, ATP degraded faster.

GRAPHIC ABSTRACT



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ABSTRACT

The efficacy of ultraviolet (UV) disinfection has been validated in numerous studies by using culture-based methods. However, the discovery of viable but non-culturable bacteria has necessitated the investigation of UV disinfection based on bacterial viability parameters. We used quantitative polymerase chain reaction (qPCR) to investigate DNA damage and evaluated adenosine triphosphate (ATP) to indicate bacterial viability. The results of qPCR effectively showed the DNA damage induced by UV when using longer gene amplicons, in that sufficiently long amplicons of both 16S and *gadA* indicated that the UV induced DNA damages. The copy concentrations of the long amplicons of 16S and *gadA* decreased by 2.38 log/mL and 1.88 log/mL, respectively, after exposure to 40 mJ/cm^2 low-pressure UV. After UV exposure, the ATP level in the bacteria did not decrease instantly. Instead it decreased gradually at a rate that was positively related to the UV fluence. For low-pressure UV, this rate of decrease was slow, but for medium pressure UV, this rate of decrease was relatively high when the UV fluence reached 40 mJ/cm^2. At the same UV fluence, the ATP level in the bacteria decreased at a faster rate after exposure to medium-pressure UV.

1 Introduction

Ultraviolet (UV) disinfection is a promising water-disinfection technology, with considerably higher effec-

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tiveness against protozoan parasites and negligible influence in terms of the formation of disinfection byproducts (Reckhow et al., 2010; Nie et al., 2017) compared with conventional oxidative chemical disinfectants (Liu et al., 2017; Wang et al., 2018). These advantages are rooted in the UV wavelength range of 10–400 nm, where UV photons have high energy owing to wave-particle duality and strong photochemical reactivity. This property allows for the use of UV radiation in many environment-related applications (Wang et al., 2017; Lu et al., 2018), including disinfection. UV radiation absorbed by nucleic acids can induce photochemical damage, thus hindering the reproduction of microorganisms (Hijnen et al., 2006).

The broad-spectrum efficacy of UV disinfection has been established in an extensive body of scientific literature and decades of safe public-health practices (Zimmer and Slawson, 2002; Lehtola et al., 2003; Murray et al., 2015). Many studies have used culture-based methods and have been based on the rationale that culturable parameters can effectively indicate the risk levels of bacterial pathogens. However, after UV treatment, microorganisms have been known to maintain their viability to a certain extent because UV radiation does not have a considerable direct effect on several viability parameters as it does on culturability parameters, including respiration rate (Blatchley et al., 2001), adenosine triphosphate (ATP) levels (Xu et al., 2018), mRNA (Yang et al., 2019), and membrane permeability (Nie et al., 2016). Therefore, it's important to select suitable analytical methods and correctly interpret the results when investigating the performance of UV disinfection. One classic example is the shift in our understanding of the effectiveness of UV radiation against *Cryptosporidium parvum* and *Giardia lamblia*. When methods based on infectivity are used, UV is considered a favorable choice for protozoan parasite control (Blatchley et al., 2017).

The microbiological phenomenon of viable but nonculturable (VBNC) bacteria (Xu et al., 1982) necessitates a review of the rationale underlying culture-based methods. Viable but nonculturable cells can not be detected using conventional culture techniques, and these cells retain different viable cell traits (Oliver, 2000). Compared with other nongrowth states, such as sporulation, persistence, and dormancy, the VBNC state is perceived as a more universal cell strategy for survival under adverse environmental conditions (Pinto et al., 2015). The ubiquity of this phenomenon emphasizes the need to inspect the efficacy of disinfection technologies, considering the possibility that the disinfectants themselves act as an adverse environmental factor that induces cells to enter this state instead of effectively inactivating them. For UV disinfection, this possibility is intuitively higher because of its DNA-damaging mechanism with little direct effect on other cellular functions.

However, because viability parameters have been used to assess UV disinfection thus far, several conflicting or insufficient results have been obtained. For example, DNA lesions appeared insignificant according to quantitative polymerase chain reaction (qPCR) results (Zhang et al., 2015), which is contrary to the accepted knowledge that UV radiation induces extensive DNA damage when an endonuclease sensitive site assay is used (Oguma et al., 2001). Another example is that when volatile ATP has been used as the viability parameter, it has usually been measured only immediately after UV irradiation (Kong et al., 2016; Xu et al., 2018). However, considering that UV radiation is not expected to directly affect the levels of ATP and damage caused by it (mostly to nucleic acids, as aforementioned) might take time to measurably influence the viability, we assumed that ATP would degrade faster after UV irradiation. Therefore, in addition to the snapshot of the ATP level after UV irradiation, the degradation of ATP should be monitored to comprehensively explain the effect of UV radiation on microbiological viability.

In this study, we investigated the UV dose responses of *Escherichia coli (E. coli)* and *staphylococcus aureus(S. aureus)* by using colony-forming units (CFU) as a culture-based parameter and ATP level as a viability parameter. In addition, we reevaluated the ability of UV radiation to damage DNA with qPCR. An appropriate methodology to measure the damage induced by UV irradiation in bacteria was developed based on ATP levels and qPCR to confirm that UV radiation induces significant DNA damage and loss of cell viability.

2 Materials and methods

2.1 Propagation and enumeration of bacteria

We selected *E. coli* (CGMCC #1.2154) and *S. aureus* (CGMCC #1.2465) as the representative gram-negative and gram-positive bacteria, respectively. Both bacteria were cultivated in nutrient broth (Aoboxing Biotechnology, China) at 37°C and shaken at 120 r/min for 16 h to ensure they enter the stationary phase. Centrifugation of the broth was performed at 5000 g at 4°C for 10 min to collect the cells, and phosphate-buffered saline (PBS) supplemented with 0.01% Tween 80 (v/v) was used to resuspend the cells. A washed cell stock of approximately 10^8 CFU/mL was prepared by repeating the centrifugation and resuspension processes twice. The cell stock was refrigerated for short-term storage. For the subsequent UV irradiation experiments, the stock was diluted 100× in PBS supplemented with 0.01% Tween 80. Therefore, the bacterial count of the microbial sample used in the UV experiments was approximately 10^6 CFU/mL. The UV absorbance of the samples between 200 and 300 nm was measured before irradiation to calculate the exposure time according to the method described in section 2.2, and the absorbance at 254 nm was slightly lower than 0.1.

Escherichia coli and *S. aureus* were enumerated in the

form of CFUs. Samples with bacterial concentrations lower than 100 CFU/mL were measured using the pour plating technique in triplicate, and samples with bacterial concentrations higher than 100 CFU/mL were measured using the spot plating technique with 10 replicates (Gaudy et al., 1963).

2.2 UV irradiation and dark incubation

Both low-pressure (LP) and medium-pressure (MP) UV irradiation experiments were conducted using collimated beam apparatuses and following the highly standardized bench-scale protocol (Bolton and Linden, 2003). In the LPUV irradiation experiments, the irradiation time was calculated using the method described in the bench-scale protocol, and the collimated beam apparatus was used with a 40 W LP mercury lamp (Philips, Netherlands) emitting 253.7 nm monochromatically. The UV intensity distribution on the sample surface was measured using a UV 254 radiometer (Photoelectric Instrument Factory of Beijing Normal University, China). The irradiance at the sample center surface ranged from 0.25 to 0.28 mW/cm^2.

In the MPUV irradiation experiments, the effective UV dose was calculated using a model (Linden and Darby, 1997) requiring the sensor sensitivity, lamp output spectrum and action spectrum, and absorbance of the microbial sample as inputs. With this model, the effective germicidal intensity relative to UV irradiation of 254 nm can be calculated at wavelength intervals of 1 nm, and the sum of the germicidal intensities at the wavelengths between 200 and 300 nm can be considered the total effective MPUV intensity. Then, the irradiation time can be calculated by dividing the predesigned UV dose with the effective MPUV intensity. In the MPUV irradiation experiments, the collimated beam apparatus was used with a 2.8 kW MP mercury vapor lamp (Philips, Netherlands), and the lamp output spectrum was measured with a spectrometer (Ocean Optics, Model Maya2000 Pro, USA). The UV irradiance at the center of the Petri dish was measured using a radiometer (International Light Technology, Model ILT7000, USA) with a calibrated UV detector (International Light Technology, Model SED240, USA), whose sensor sensitivity was specified by the manufacturer. When we used a sensor factor for the UV irradiation of 254 nm, the irradiance reading at the center of the sample surface was approximately 0.16 mW/cm^2. While this reading was not the actual intensity, it was input directly into the model. The absorbance spectrum of DNA (Rauth, 1965) was used as a surrogate to the action spectrum, which is an acceptable practice given the similarity between the UV action spectrum and the DNA absorbance spectrum (Pirnie et al., 2006). The absorbance of the microbial sample was measured as described in section 2.1.

In the LPUV or the MPUV irradiation experiment, the lamps were turned on at least 30 min before commencing the experiments and measurements. Under the beam, a stir plate was used to stir the samples continuously for maintaining homogeneity. The samples were exposed to UV doses of 5, 10, 20, 40, and 80 mJ/cm^2, and the exposure time ranged between 30 s and 16 min. Irradiation was conducted at room temperature. For each exposure, 40 mL of the sample was prepared in a 90-mm Petri dish containing a small sterile magnetic bar. The samples were kept in the dark, except when being irradiated, to limit the potential repair effect of ambient light. Two independent UV irradiation experiments were conducted for each UV dose.

Dark incubation was performed to investigate ATP degradation after UV irradiation. The samples were transferred to 50-mL capped centrifuge tubes and shaken at 100 r/min at room temperature.

2.3 DNA extraction and qPCR

We used 40 mL of the bacterial sample, prepared as described in the previous section, was used for DNA extraction after centrifuging (8000 g at 4°C for 10 min) and discarding the supernatant. This process was repeated thrice to concentrate the bacteria. Thus, 120 mL of the bacterial sample was used for one DNA extraction. The pellet was resuspended in 0.5 mL of PBS. The EZNA® Soil DNA extraction kit (Omega Bio-Tek, USA) was used to extract DNA from this 0.5 mL sample following the manufacturer's protocol, and 50 μL of DNA solution was generated. The concentration and purity of the extracted DNA were measured using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

The qPCR method was used to analyze the DNA samples. The amplified target genes and the primers used in this study are listed in Table 1. A LightCycler 96 System (Roche, Germany) and the FastStart Essential DNA Green Master hot start reaction mix (Roche, Germany) were used according to the manufacturer's instructions to perform qPCR. We used the following recipe to prepare 20 μL of reaction-ready solution: 10 μL of master mix, 2 μL of PCR

Table 1 The amplified target genes and the primers used in this study

Species	Target gene	Primer sequence (F/R)	Product size
E. coli	16S short	CCTACGGGAGGCAGCAG/ATTACCGCGGCTGCTGG	194
E. coli	16S long	AGAGTTTGATCCTGGCTCAG/TACGGCTACCTTGTTACGACTT	1465
E. coli	*gadA* short	GGTGATGCGCATTATGTGTC/CGGGTGATCGCTGAGATATT	100
E. coli	*gadA* long	GGTTCTTCCGAGGCCTGTAT/CATAATGCGCATCACCACGA	900

primer, 3 μL of PCR-grade water, and 5 μL of DNA template. The reaction parameters of the qPCR procedure were as follows: pre-incubation at 95°C for 5 min; amplification of 45 cycles with each cycle being 95°C for 15 s, 60°C for 45 s, and 72°C for 30 s sequentially; melt curve generation at 95°C for 10 s, and 55°C for 60 s; and heating to 97°C at a rate of 0.1°C/s. For amplification of the 16S long and the *gadA* long gene amplicons, the duration of the 72°C elongation step during the amplification cycle was calculated by dividing the amplicon length with 10, according to the manufacturer's instructions; specifically, the values were 150 s for 16S long amplicon and 95 s for the *gadA* long amplicon. The standard DNA templates were provided and quantified by Geneway (China). These templates were used to generate a standard curve for absolute quantification of the gene copy number in each sample.

2.4 ATP assay

After the disinfection experiments, the ATP levels of both *E. coli* and *S. aureus* were measured using the BacTiter–Glo™ Microbial Cell Viability Assay kit (G8232, Promega, USA). The assay was performed according to the manufacturer's protocol. The reagent was prepared by equilibrating the buffer and substrate to room temperature before the experiment, following by mixing both to form the active reagent. We added 100 μL of the sample to one well of an opaque-walled 96-well plate, together with a 10-fold serial dilution of standard ATP (A2383, Sigma, USA). The same volume (100 μL) of reagent was then added to each well. An orbital shaker was used to mix the reagent and sample at room temperature for 5 min. Luminescence was measured using the SpectraMax i3 Multi-Mode Detection Platform (Molecular Devices, USA). The ATP concentration of the samples was calculated using a standard curve for ATP.

3 Results and discussion

3.1 Effect of UV disinfection on the culturability of *E. coli* and *S. aureus*

The UV dose response curves of *E. coli* and *S. aureus* based on CFUs are shown in Fig. 1. Inactivation reached 5.21 log and 5.30 log for LPUV irradiation with a fluence of 20 mJ/cm^2 and 5.10 log and 4.40 log for MPUV irradiation with a fluence of 20 mJ/cm^2 for *E. coli* and *S. aureus*, respectively. After 20 mJ/cm^2, all response curves entered a lag phase, reaching 7.15 log and 5.93 log for LPUV irradiation with a fluence of 80 mJ/cm^2 and 6.27 log and 5.39 log for MPUV irradiation with a fluence of 80 mJ/cm^2 for *E. coli* and *S. aureus*, respectively.

The results of this study are consistent with the common knowledge that both LPUV and MPUV irradiation are highly effective for inactivating both gram-negative and gram-positive bacteria. The UV response curves of two representative bacteria are similar to those obtained in other studies on UV disinfection, and although there are variations among the microbial responses of different microorganisms, bacteria can effectively be inactivated with a UV fluence of no more than 40 mJ/cm^2 (Sommer et al., 1998; Sommer et al., 2000; Guo et al., 2009; Fang et al., 2014; Blatchley et al., 2017). The differences between LPUV and MPUV irradiation were limited because the MPUV doses were reported in terms of the effective germicidal dose normalized to 254 nm, which is essentially LPUV irradiation.

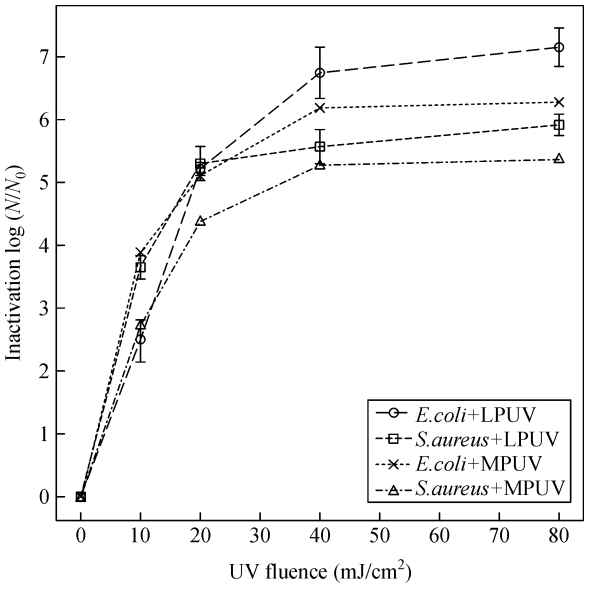

Fig. 1 UV response curves of *Escherichia coli* and *Staphylococcus aureus*.

3.2 Using qPCR to measure the effect of UV disinfection on DNA

The main mechanism of UV disinfection of DNA involves the induction of lesions and the interruption of genome replication, thereby terminating cell cycle progression (Jagger, 1967; Snowball and Hornsey, 1988). Theoretically, qPCR should be able to detect UV damages because these damages can hinder the extension process and reduce the efficiency of the PCR, and therefore, attenuate the qPCR signals (Smith et al., 1998; Ayala-Torres et al., 2000). In this study, the effects of UV on DNA were investigated by measuring the qPCR signals before and after UV irradiation. The *E. coli* and the primer sets used were the same as those used by Zhang et al. (2015). The LPUV mercury lamp was selected as the sole UV source because its monochromatic output induces only DNA damages, whereas the polychromatic output of the MPUV mercury lamp can induce protein damages as well

(Eischeid et al., 2011) and may introduce unnecessary variations. The values of copy number/mL of bacteria sample are shown in Fig. 2.

For the 16S and *gadA* genes, the long and short amplicons of each gene exhibited log differences of less than 1 at 0 mJ/cm^2. This result was expected because the DNA in the untreated samples was not damaged, which means amplicons of different lengths should exhibit the same copy number. The shorter gene amplicons were less sensitive in detecting DNA lesions because the copy number decreased at a slower rate (as in the case of *gadA*) or the decrease was not consistent (as in the case of 16S). The longer gene amplicons showed greater sensitivity in detecting DNA lesions and the rate of decrease of the copy number/mL showed a stronger correlation with the UV fluence. For the UV fluence of 40 mJ/cm^2, the copy number of the long amplicons decreased by approximately 2 log.

A theoretical framework developed by Pecson et al. (2011) can explain this phenomenon more explicitly. Assuming that the UV damage follows the Poisson distribution, the "detected" undamaged proportion of DNA can be calculated using the following equation (Pecson et al., 2011):

$$\text{"}Detected\text{" } undamaged\ proportion = e^{-Total\ genome\ lesions \cdot Amplicon\ size/Genome\ size}, \quad (1)$$

The total genome lesions and genome size should be considered constant for a given bacterium and UV fluence. Thus, the "detected" undamaged proportion is related negatively to amplicon size. For longer amplicons, the "detected" undamaged proportion will be smaller, that is, the decrease in copy number will be steeper. It was recently reported that the faster qPCR signal loss of longer amplicons after UV exposure can be majorly accounted for by thymine–thymine dimers (He et al., 2019). For both 16S and *gadA*, the copy numbers of the long and short amplicons were the same at 0 mJ/cm^2. At the UV fluence of 40 mJ/cm^2, the copy numbers of the long and short amplicons of 16S decreased by 2.38 log/mL and 1.45 log/mL, respectively, and those of *gadA* decreased by 1.88 log/mL and 0.45 log/mL. According to these two-point decreases, the sensitivities of the qPCR amplicons in "detecting" DNA damage were ranked as follows: *gadA* short < 16S short < *gadA* long < 16S long. This sequence is identical to the sequence ordered by amplicon length.

3.3 Effect of UV disinfection on ATP degradation in *E. coli* and *S. aureus*

In this study, we investigated the effects of LPUV and MPUV irradiation on the ATP in *E. coli* and *S. aureus*. We measured the ATP level within 15 min of exposure. The results are reported as a percentage of the concentration in unexposed samples (Fig. 3). The ATP levels of *E. coli* and *S. aureus* generally decreased as the UV fluence increased. This decrease reached up to approximately 20% at 80 mJ/cm^2.

Here, we assumed that the ATP in UV-exposed bacteria would degrade faster as a result of the DNA lesions induced. To test this assumption, bacterial samples were placed in the dark after UV irradiation, and their ATP levels were tested. For convenience, the ATP concentration of a

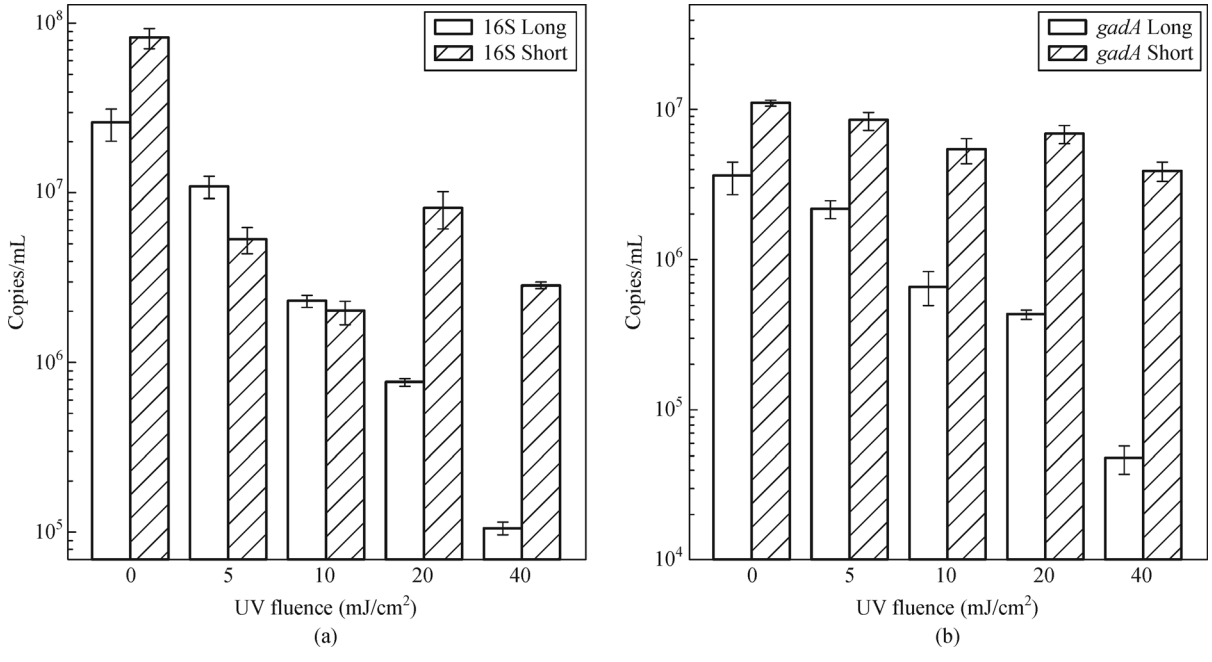

Fig. 2 Copy numbers of long and short gene amplicons in *Escherichia coli* after low pressure UV irradiation based on qPCR. (a) 16S; (b) *gadA*.

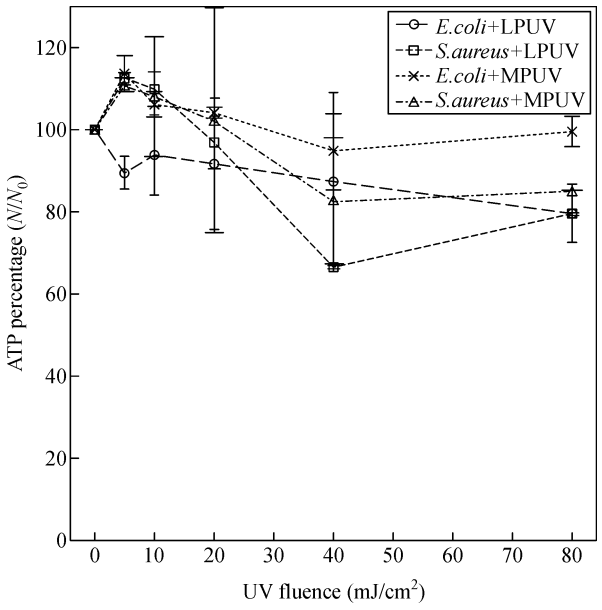

Fig. 3 Effects of LPUV and MPUV irradiation on ATP levels of *Escherichia coli* and *Staphylococcus aureus.*

sample exposed to X mJ/cm^2 UV for T hours of dark incubation was denoted by ATP(X, T).

ATP(0, T)/ATP(0, 0)(%) values are shown in Fig. 4 to demonstrate the natural degradation of ATP in *E. coli* and *S. aureus*. The ATP standard, diluted in ultrapure water, gradually degraded to 62.7% of its original concentration after 48 h of incubation in the dark, presumably because of the unstable nature of ATP. The ATP in *S. aureus* showed a similar pattern of decrease as that of the pure ATP sample. However, the ATP in *E. coli* degraded at a higher rate, and

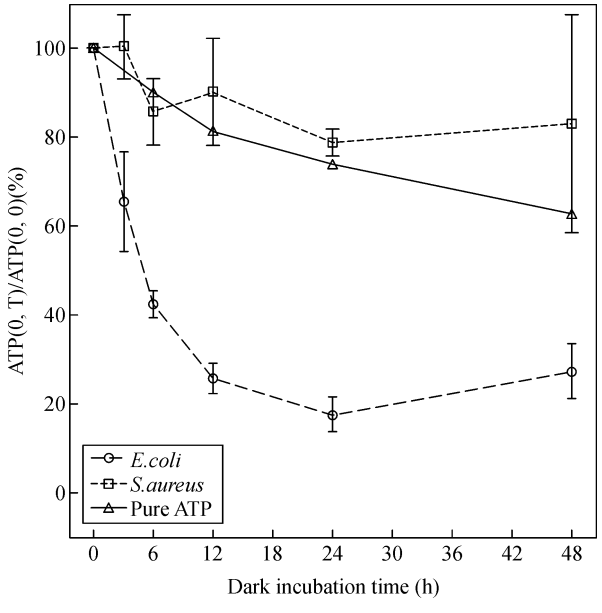

Fig. 4 Natural degradation of pure ATP samples and the ATP in *Escherichia coli* and *Staphylococcus aureus.*

the ATP concentration decreased to 27.4% of the original concentration.

The samples exposed to different UV fluences were incubated in the same dark environment. At each time point, the ATP(X, T)/ATP(0, T)(%) value indicates the UV-induced degradation of the ATP in *E. coli* and *S. aureus*. Assuming that the degradation rate of ATP follows first-order reaction kinetics, meaning ATP(0, T) = ATP(0, 0)$\cdot e^{-\lambda(0)T}$, ATP(X, T) = ATP(X, 0)$\cdot e^{-\lambda(X)T}$, then ATP(X, T)/ATP(0, T) = (ATP(X, 0)/ATP(0, 0))$\cdot e^{-(\lambda(X)-\lambda(0))T} = ke^{-\Delta\lambda T}$. $\Delta\lambda$ is used to indicate the change in the rate of degradation due to UV damage. ATP(X, T)/ATP(0, T)(%) values after LPUV and MPUV exposure are shown in Figs. 5 and 6 respectively, in which the thicker lines indicate the exponential model in the form of $ke^{-\lambda T}$ fitted to each UV fluence.

After LPUV exposure, the ATP concentration in bacteria decreased gradually over time and decreased more rapidly when exposed to higher UV doses. This trend was more distinct for *E. coli* because the curves representing the different UV fluences showed no crossover. By 48 h, the ATP concentration decreased further in the UV-exposed *E. coli* to 47.3%, 33.5%, 22.9%, 14.8%, and 10.8% for UV fluences of 5, 10, 20, 40, and 80 mJ/cm^2, respectively. Greater variation was observed in the case of *S. aureus* because the curves were more clustered and not strictly aligned monotonically. By 48 h, the ATP concentration decreased further in the UV-exposed *S. aureus* to 31.9%, 36.4%, 17.6%, 14.6%, and 14.1% for UV fluences of 5, 10, 20, 40, and 80 mJ/cm^2, respectively.

The standard deviations within 12 h are large. This was attributed to the inherent difficulty associated with experiment design because of rapid ATP degradation, uniformity requirement of the ATP reagent, and limited capacity of the UV irradiation apparatus. The samples were tested immediately after sequential UV irradiation to demonstrate the minimum effect of UV on ATP, as shown in Fig. 3, because of which the starting times could not be aligned perfectly. The longest duration of UV irradiation, which was approximately 16 min for the fluence 80 mJ/cm^2, can be considered the time difference. For all time points other than 0 h, there is a trade-off where either the ATP experiments were performed in batches to achieve uniformity in ATP reagent reaction, in which case the dark incubation time difference was introduced, or the dark incubation time was recorded precisely, in which case the quality of the ATP experiment was compromised. In this study, the former approach was adopted because the standard deviation converged better after 24 h, and the phenomenon was believed to be demonstrated adequately.

After MPUV exposure, in addition to a decrease pattern similar to that observed for LPUV exposure, the most noteworthy phenomenon was observed at the UV fluence of 40 mJ/cm^2. At this dose, the ATP decrease was greatest after just 3 h of dark incubation, after which the decrease entered a lag phase. In *E. coli*, the ATP concentration

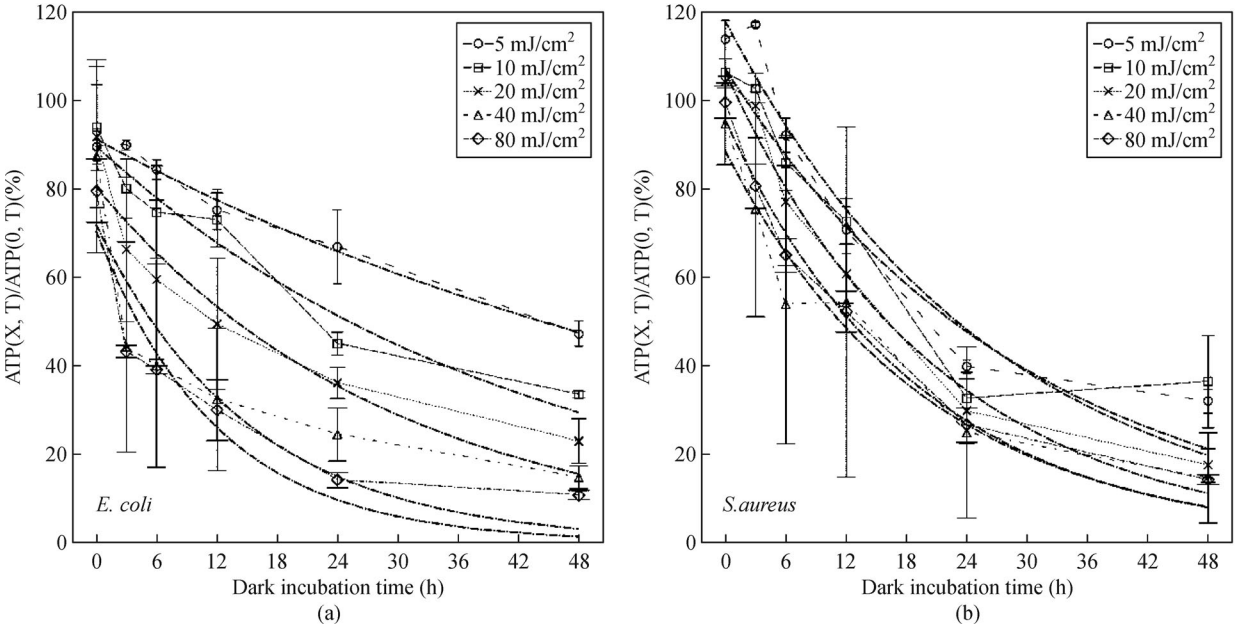

Fig. 5 LPUV-induced additional degradation of ATP in (a) *Escherichia coli* and (b) *Staphylococcus aureus*

decreased further to 3.6% and 3.9% for the fluences of 40 and 80 mJ/cm^2, respectively, after 3 h and to 16.2%, 9.5%, 4.6%, 2.8%, and 2.4% for the fluences of 5, 10, 20, 40, and 80 mJ/cm^2, respectively, after 48 h. In *S. aureus,* the ATP concentration decreased further to 3.5% and 1.4% for the fluences of 40 and 80 mJ/cm^2, respectively, after 3 h and to 31.7%, 13.1%, 8.0%, 5.9%, and 5.4% for the fluences of 5, 10, 20, 40, and 80 mJ/cm^2, respectively, after 48 h.

Recent studies have suggested that UV does not have a significant effect on ATP (Kong et al., 2016; Xu et al., 2018). At a UV fluence of 100 mJ/cm^2, the ATP concentration in *E. coli* decreased by 8.56% (Xu et al., 2018). According to Kong et al. (2016), UV had little effect on the ATP concentration in *E. coli*, while it lowered the ATP concentration in *Bacillus subtilis* by as much as 2.5 log at 80 mJ/cm^2. The results presented in Fig. 3 are similar to the results obtained for bacteria (rather than spores) in the studies cited earlier in this paragraph. The ATP concentration did decrease after UV exposure, but the effect was negligible compared with the efficacy measured in terms of CFU. Moreover, there were no apparent differences in terms of the bacterial species or type of UV light source. However, this result was expected because UV is not known to induce ATP degradation directly.

The assumption that the ATP in UV-exposed bacteria degrades faster can be proved by the results presented in Figs. 5 and 6. The degradation shown in these two figures does not include the natural degradation shown in Fig. 4 because ATP(X, T) has been normalized by ATP(0, T). Therefore the decreases in Figs. 5 and 6 indicates solely the "additional" ATP degradation induced by UV damage, and the declining trends prove that UV accelerates ATP degradation.

The additional ATP degradation occurs at a faster rate after exposure to higher UV fluences, as well as after exposure to MPUV irradiation rather than LPUV irradiation. It is more convenient to use the fitting model for further discussion. The parameters k, $\Delta\lambda$, and r^2 of the fitted model are listed in Table 2. The r^2 values of all but three of the models were higher than 0.9, indicating a good fit between this model and the pattern of additional ATP degradation induced by LPUV or MPUV irradiation. The parameter k represents ATP(X, 0)/ATP(0, 0), and in this study, its value was higher than 0.7 in all cases. These results are consistent with those in Fig. 3, indicating that UV does not induce instant ATP degradation.

As for $\Delta\lambda$, a significant difference was observed between LPUV and MPUV irradiation, while the difference between the two species of bacteria was not significant. As the UV fluence increased, the $\Delta\lambda$ of LPUV irradiation for either *E. coli* or *S. aureus* increased gradually but never exceeded 0.1. The $\Delta\lambda$ of MPUV irradiation started at a similar level as that of LPUV irradiation at the fluence of 5 mJ/cm^2 but reached 1.0 at the fluence of 40 mJ/cm^2. For both *E. coli* and *S. aureus*, further UV-induced ATP degradation occurred at a significantly faster rate under irradiation with the MPUV light source than that with the LPUV light source. While LPUV radiation is monochromatic and only damages nucleic acids, MPUV radiation is polychromatic and can damage both nucleic acids and proteins. Protein damage could theoretically lead to a collapse of intracellular systems, thus causing a shift in the equilibrium between energy production and consumption. Considering that severe cell damage eventually causes cell death, and the ATP level is the minimum in a dead cell, the above equilibrium shift is more likely to reduce the ATP level.

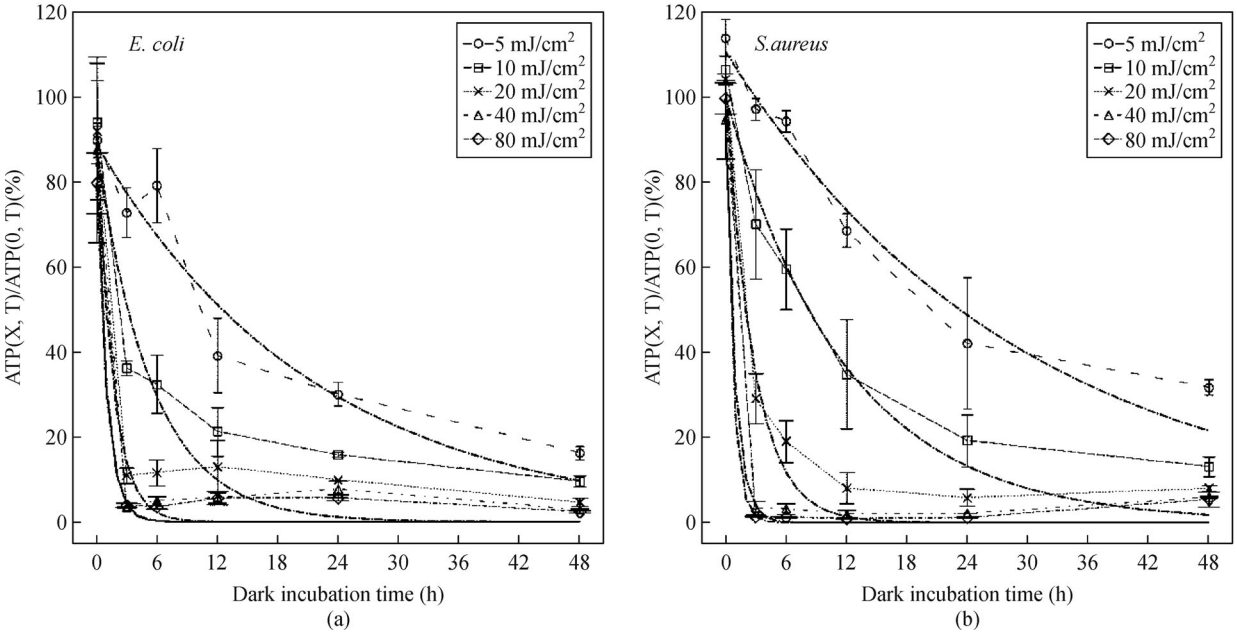

Fig. 6 MPUV-induced additional degradation of ATP in (a) *Escherichia coli* and (b) *Staphylococcus aureus.*

Table 2 The fitted exponential degradation model $ke^{-\lambda T}$ of ATP(X, T)/ATP(0, T)(%) for *Escherichia coli* and *Staphylococcus aureus* under different LPUV and MPUV fluences

UV fluence (mJ/cm^2)	*E. coli*		*S.aureus*	
	LPUV	MPUV	LPUV	MPUV
5	$0.910 \cdot e^{-0.014T}$, $r^2 = 0.989$	$0.885 \cdot e^{-0.046T}$, $r^2 = 0.923$	$1.178 \cdot e^{-0.037T}$, $r^2 = 0.940$	$1.104 \cdot e^{-0.034T}$, $r^2 = 0.961$
10	$0.894 \cdot e^{-0.023T}$, $r^2 = 0.952$	$0.877 \cdot e^{-0.182T}$, $r^2 = 0.852$	$1.071 \cdot e^{-0.034T}$, $r^2 = 0.903$	$1.000 \cdot e^{-0.084T}$, $r^2 = 0.957$
20	$0.803 \cdot e^{-0.034T}$, $r^2 = 0.906$	$0.914 \cdot e^{-0.625T}$, $r^2 = 0.931$	$1.064 \cdot e^{-0.047T}$, $r^2 = 0.981$	$1.029 \cdot e^{-0.360T}$, $r^2 = 0.968$
40	$0.718 \cdot e^{-0.066T}$, $r^2 = 0.762$	$0.872 \cdot e^{-1.027T}$, $r^2 = 0.978$	$0.884 \cdot e^{-0.050T}$, $r^2 = 0.943$	$0.945 \cdot e^{-1.080T}$, $r^2 = 0.992$
80	$0.703 \cdot e^{-0.083T}$, $r^2 = 0.884$	$0.795 \cdot e^{-0.973T}$, $r^2 = 0.983$	$0.955 \cdot e^{-0.052T}$, $r^2 = 0.984$	$0.994 \cdot e^{-1.413T}$, $r^2 = 0.996$

This can explain the rapid ATP damage in bacteria upon exposure to MPUV radiation relative to that upon exposure to LPUV radiation. However, when *S. aureus* was exposed to LPUV or MPUV radiation, a few datapoints in Figs. 5 and 6 are higher than 100% at 0 h, indicating that the ATP might increase temporarily owing to a more active response system. The results in this study were not conclusive to validate this hypothesis though.

4 Conclusions

UV disinfection has a unique mechanism of inducing DNA damage without directly interrupting other cellular functions. Because of this mechanism, selection of the experimental technique and experimental design is critical. This study showed that UV is effective for reducing bacterial culturability, and qPCR can effectively be used to measure the UV-induced DNA damage by using longer gene amplicons. Moreover, when adequately long amplicons of both the 16S and *gadA* genes are selected, both genes can be used as markers of UV-induced DNA damage. After UV exposure, the ATP in bacteria does not degrade instantly but does so eventually, indicating that UV can affect bacterial viability. Under LPUV irradiation, the ATP in bacteria decreased gradually. By contrast, under MPUV irradiation with fluences higher than 40 mJ/cm^2, the ATP in bacteria decreased rather rapidly. Medium-pressure UV irradiation induces faster ATP degradation compared with LPUV irradiation at the same fluence. Thus, although the efficacies of UV disinfection with LPUV and MPUV irradiation did not differ significantly when using a culture-based method, bacteria seemed to lose viability more rapidly under MPUV exposure. As a reference for studies on viability parameters similar to ATP, the temporary stability of a viability parameter might be inadequate for indicating that UV induces bacteria into the VBNC state.

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References

Ayala-Torres S, Chen Y, Svoboda T, Rosenblatt J, Van Houten B (2000). Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction. Methods (San Diego, Calif.), 22(2): 135–147

Blatchley E R 3rd, Dumoutier N, Halaby T N, Levi Y, Laîné J M (2001). Bacterial responses to ultraviolet irradiation. Water Science and Technology, 43(10): 179–186

Blatchley E R, Oguma K, Sommer R (2017). Comment on 'UV disinfection induces a VBNC state in *Escherichia coli* and *Pseudomonas aeruginosa*'. IUVA News, 18(3): 12–16

Bolton J R, Linden K G (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. Journal of Environmental Engineering, 129(3): 209–215

Eischeid A C,Thurston J A, Linden K G (2011). UV disinfection of adenovirus: present state of the research and future directions. Critical Reviews in Environmental Science and Technology, 41(15): 1375–1396

Fang J, Liu H, Shang C, Zeng M, Ni M, Liu W (2014). *E. coli* and bacteriophage MS2 disinfection by UV, ozone and the combined UV and ozone processes. Frontiers of Environmental Science & Engineering, 8(4): 547–552

Gaudy A F Jr, Abu-Niaaj F, Gaudy E T (1963). Statistical study of the spot-plate technique for viable-cell counts. Applied Microbiology, 11 (4): 305–309

Guo M, Hu H, Bolton J R, El-Din M G (2009). Comparison of low- and medium-pressure ultraviolet lamps: Photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. Water Research, 43(3): 815–821

He H, Zhou P, Shimabuku K K, Fang X, Li S, Lee Y, Dodd M C (2019). Degradation and deactivation of bacterial antibiotic resistance genes during exposure to free chlorine, monochloramine, chlorine dioxide, ozone, ultraviolet light, and hydroxyl radical. Environmental Science & Technology, 53(4): 2013–2026

Hijnen W A, Beerendonk E F, Medema G J (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. Water Research, 40(1): 3–22

Jagger J (1967). Introduction to Research in Ultraviolet Photobiology. Englewood Cliffs, NJ: Prentice-Hall

Kong X, Ma J, Wen G, Wei Y (2016). Considerable discrepancies among HPC, ATP, and FCM detection methods in evaluating the disinfection efficiency of Gram-positive and-negative bacterium by ultraviolet radiation and chlorination. Desalination and Water Treatment, 57 (37): 17537–17546

Lehtola M J, Miettinen I T, Vartiainen T, Rantakokko P, Hirvonen A, Martikainen P J (2003). Impact of UV disinfection on microbially available phosphorus, organic carbon, and microbial growth in drinking water. Water Research, 37(5): 1064–1070

Linden K G, Darby J L (1997). Estimating effective germicidal dose from medium pressure UV lamps. Journal of Environmental Engineering, 123(11): 1142–1149

Liu Y, Zhang Q, Hong Y (2017). Formation of disinfection byproducts from accumulated soluble products of oleaginous microalga after chlorination. Frontiers of Environmental Science & Engineering, 11 (6): 1

Lu S, Wang N, Wang C (2018). Oxidation and biotoxicity assessment of microcystin-LR using different AOPs based on UV, O_3 and H_2O_2. Frontiers of Environmental Science & Engineering, 12(3): 12

Murray K E, Manitou-Alvarez E I, Inniss E C, Healy F G, Bodour A A (2015). Assessment of oxidative and UV-C treatments for inactivating bacterial biofilms from groundwater wells. Frontiers of Environmental Science & Engineering, 9(1): 39–49

Nie X, Liu W, Chen M, Liu M, Ao L (2016). Flow cytometric assessment of the effects of chlorine, chloramine, and UV on bacteria by using nucleic acid stains and 5-cyano-2,3-ditolyltetrazolium chloride. Frontiers of Environmental Science & Engineering, 10(6): 12

Nie X, Liu W, Zhang L, Liu Q (2017). Genotoxicity of drinking water treated with different disinfectants and effects of disinfection conditions detected by umu-test. Journal of Environmental Sciences-China, 56: 36–44

Oguma K, Katayama H, Mitani H, Morita S, Hirata T, Ohgaki S (2001). Determination of pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation, photoreactivation, and dark repair. Applied and Environmental Microbiology, 67 (10): 4630–4637

Oliver J D (2000). The Public Health Significance of Viable but Nonculturable Bacteria. Boston: Springer, 277–300

Pecson B M, Ackermann M, Kohn T (2011). Framework for using quantitative PCR as a nonculture based method to estimate virus infectivity. Environmental Science & Technology, 45(6): 2257–2263

Pinto D, Santos M A, Chambel L (2015). Thirty years of viable but nonculturable state research: Unsolved molecular mechanisms. Critical Reviews in Microbiology, 41(1): 61–76

Pirnie M, Linden K G, Malley J P, Schmelling D, Usa O O W (2006). Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule: EPA 815-R-06–007. Washington, DC: EPA, 2–8

Rauth A M (1965). The physical state of viral nucleic acid and the sensitivity of viruses to ultraviolet light. Biophysical Journal, 5(3): 257–273

Reckhow D A, Linden K G, Kim J, Shemer H, Makdissy G (2010). Effect of UV treatment on DBP formation. Journal-American Water Works Association, 102(6): 100–113

Smith C A, Baeten J, Taylor J S (1998). The ability of a variety of polymerases to synthesize past site-specific *cis-syn*, *trans-syn*-II, (6-4), and Dewar photoproducts of thymidylyl-(3′ → 5′)-thymidine. Journal of Biological Chemistry, 273(34): 21933–21940

Snowball M R, Hornsey I S (1988). Purification of water supplies using ultraviolet light. Developments in Food Microbiology, 3: 171–191

Sommer R, Haider T, Cabaj A, Pribil W, Lhotsky M (1998). Time dose reciprocity in UV disinfection of water. Water Science and Technology, 38(12): 145–150

Sommer R, Lhotsky M, Haider T, Cabaj A (2000). UV inactivation, liquid-holding recovery, and photoreactivation of Escherichia coli O157 and other pathogenic Escherichia coli strains in water. Journal of Food Protection, 63(8): 1015–1020

Wang F, Li W, Li Y, Zhang J, Chen J, Zhang W, Wu X (2018). Molecular analysis of bacterial community in the tap water with different water ages of a drinking water distribution system. Frontiers of Environmental Science & Engineering, 12(3): 6

Wang N Y, Wang K, Wang C (2017). Comparison of different algicides on growth of Microcystis aeruginosa and microcystin release, as well as its removal pathway in riverways. Frontiers of Environmental Science & Engineering, 11(6): 3

Xu H S, Roberts N, Singleton F L, Attwell R W, Grimes D J, Colwell R R (1982). Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. Microbial Ecology, 8(4): 313–323

Xu L, Zhang C, Xu P, Wang X C (2018). Mechanisms of ultraviolet disinfection and chlorination of *Escherichia coli*: Culturability, membrane permeability, metabolism, and genetic damage. Journal of Environmental Sciences-China, 65: 356–366

Yang C, Sun W, Ao X (2019). Using mRNA to investigate the effect of low-pressure ultraviolet disinfection on the viability of *E. coli*. Frontiers of Environmental Science & Engineering, 13(2): 26

Zhang S, Ye C, Lin H, Lv L, Yu X (2015). UV disinfection induces a VBNC state in *Escherichia coli* and *Pseudomonas aeruginosa.* Environmental Science & Technology, 49(3): 1721–1728

Zimmer J L, Slawson R M (2002). Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. Applied and Environmental Microbiology, 68(7): 3293–3299