Inactivation and Photorepair of Enteric Pathogenic Microorganisms with Ultraviolet Irradiation

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Abstract

Ultraviolet inactivation of enteric pathogenic microorganisms, including *Shigella dysenteriae*, *Salmonella typhimurium*, and human rotavirus (HRV-Wa) and somatic coliphages (MS2 and T4), was investigated in different water samples. Significant differences in the sensitivity of the bacteria and viruses to ultraviolet radiation were observed. Viruses were more resistant to ultraviolet disinfection than all the enteric bacteria tested in this study. With the exception of *S. typhimurium*, which showed flattening and tailing, strong first-order relationships between the logarithm of survival and the ultraviolet dose were observed. While *S. dysenteriae* and *S. typhimurium* were found to undergo photoreactivation after ultraviolet exposure, the photoreactivation decreased significantly with higher ultraviolet doses. Moreover, the inactivated bacteria exhibited different photoreactivation rates with different water samples. Combination of a low ultraviolet dose and a low concentration of chlorine not only inhibited the photoreactivation of ultraviolet-damaged bacteria but also effectively inactivated the bacteria.

Key words: UV disinfection; photoreactivation; chlorine; enteric pathogenic bacteria; inactivation.

Introduction

WATERBORNE PATHOGENS HAVE a devastating effect on public health. Intestinal parasitic infections and diarrheal diseases caused by waterborne bacteria and enteric viruses are a leading cause of malnutrition, since food eaten by people sickened by waterborne pathogens is poorly digested (Lima *et al.*, 2000; Montgomery and Elimelech, 2007; Shannon *et al.*, 2008). Traditional water disinfection processes rely heavily on chemical disinfectants, such as chlorine, which produce chemical disinfection by-products in finished drinking water (Muellner *et al.*, 2007). Because it does not produce chemical disinfection by-products and has excellent biocidal properties, ultraviolet (UV) disinfection is now highly regarded as an alternative to chlorination for the treatment of drinking water (Quek and Hu, 2008; Wolfe, 1990). However, because UV radiation from the sun is present in the environment, natural defense mechanisms have evolved in bacteria and other microorganisms that allow UV-inactivated microorganisms to reverse UV-induced damage through such repair pathways as photoreactivation and dark

repair (Koivunen and Heinonen-Tanski, 2005; Quek and Hu, 2008). Since these defense mechanisms are obstacles to safe disinfection levels and the application of UV disinfection, scientists have studied photoreactivation and dark repair following UV treatment extensively in the past few decades (Quek and Hu, 2008; Quek *et al.*, 2006). Just as different strains of *Escherichia coli* have different repair abilities, different types of bacteria exhibit different sensitivities to UV applications and have different repair abilities. A microorganism's UV resistance depends on the UV dose applied and its ability to protect itself from UV light and to repair damages (Sommer *et al.*, 2001). It seems clear that knowledge of the UV sensitivity of important health-related microorganisms will lead to improvements in the design of future UV processes.

Because waterborne pathogenic *Salmonella typhimurium*, *Shigella dysenteriae*, and human rotavirus (HRV-Wa) can cause bloody diarrhea and death, they pose a severe public health risk. There have been, however, very few studies of the inactivation of these bacteria by UV light and the ability of the bacteria to repair themselves after exposure. For this study, *S. typhimurium*, *S. dysenteriae*, and rotavirus were selected to evaluate UV disinfection. Their inactivation kinetics and photorepair characteristics in different water samples were investigated, and the use of chlorine to inhibit photoreactivation was evaluated. As the final step, a UV-based disinfection process was proposed.

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TABLE 1. MAIN CHEMICAL CHARACTERISTICS OF TEST WATER SAMPLES

Parameter	pH	DOC (mg/L)	UV _{254nm} (cm ⁻¹)	Cl ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	NO ₃ ⁻ (mg/L)	Alkalinity (HCO ₃ ⁻ , mM)
PBS	7.2	-	-	-	-	-	-
DW	8.00	1.90	0.0230	12.6	34.8	7.48	1.50
SW	8.25	4.60	0.0525	12.4	42.0	3.30	2.80

Materials and Methods

Preparation and enumeration of microorganisms

Bacteriophage MS2 and the bacterial host strain *E. coli* (ATCC 15597) were purchased from the American Type Culture Collection. Bacteriophage T4 and the bacterial host cells *E. coli* B were provided by the Wuhan Institute of Virology, Chinese Academy of Sciences. Enteropathogenic bacteria *S. dysenteriae* and *S. typhimurium* were obtained from the Institute of Microbiology, Chinese Academy of Sciences.

E. coli and *S. dysenteriae* were inoculated into a nutrient broth medium, while *S. typhimurium* was maintained on tryptone soy broth (TSB) (Hu *et al.*, 2010). HRV-Wa stocks were propagated, concentrated, and purified, using methods previously described by Jean *et al.* (2002). Propagation of MS2 and T4 coliphage was accomplished by the double-layer agar technique (Simonet *et al.*, 2006).

During the experiment, an aliquot of the reaction suspensions was withdrawn at various reaction times. The bacteria were immediately diluted with a 0.9% saline solution, while bacteriophages were diluted with phosphate buffered saline (PBS) and rotavirus were diluted with Dulbecco's modified Eagle medium (DMEM). The bacterial concentration of *S. dysenteriae*, *S. typhimurium*, and *E. coli* were determined by the spread plate method after 48 hours of incubation at 37°C. After incubation, the plates were counted, and the counts were averaged and recorded as colony-forming units per milliliter (CFU/mL). Infectivity titers of HRV-Wa were enumerated by determining the 50% tissue culture infectious dose (TCID₅₀), using the method described by Jean *et al.* (2002). The viable concentration of bacteriophages MS2 and T4 was achieved using the double agar layer method (Simonet *et al.*, 2006). All counts were done in duplicate.

Water samples

Local surface water (SW) was collected from the Miyun reservoir in Beijing. Treated water samples were obtained from a local municipal drinking water treatment plant (DW) in Beijing at a sampling point after sand filtration. Samples were filtered through a 0.45 µm nylon membrane, and stored at 4°C prior to use. The UV-inactivation of microorganisms was accomplished in PBS (0.01 mol/L, pH 7.2). The water quality of the three water matrices is presented in Table 1.

Experimental procedures

UV irradiation. The UV disinfection experiments were performed with a bench-scale collimated beam apparatus, consisting of a low-pressure mercury lamp that produced UV light at a wavelength of 253.7 nm (11 W, Beijing Lighting Research Institute). Sterile 60 mm petri dishes were filled with a 20 mL suspension of the microorganisms in different water samples. The suspensions were stirred with a magnetic stirrer

while being exposed to UV light. Exposure times were calculated based on the UV dose each suspension required and the average intensity of the radiation (Bolton and Linden, 2003). At the appropriate times, 0.5 mL of suspension was removed to determine the bacteria concentration.

Photoreactivation. For photoreactivation, the petri dishes containing the UV-irradiated bacteria were covered and stirred continuously under a fluorescent lamp with a light intensity of 0.066 mW/cm² at a wavelength of 360 nm for 3 hours. The percentage of photoreactivation was computed, as defined below:

$$\text{percentage photoreactivation (\%)} = \frac{\text{No. of cells photoreactivated}}{\text{No. of cells inactivated by UV}} \times 100\%$$

Inhibition of photoreactivation. After UV irradiation, a desired concentration of chlorine (1 mg/L to 2 mg/L) was immediately added to the samples, and the treated samples were then placed under fluorescent light for 3 to 4 hours. All experiments were repeated three times at room temperature (25°C ± 2°C).

Results and Discussion

UV inactivation of enteric pathogenic microorganisms

Figure 1 shows the inactivation kinetics of the various microorganisms following UV disinfection in PBS at room temperature. Because the analysis of HRV-Wa is a complex process, somatic coliphages MS2 and T4 were selected as the model viruses. As shown in Fig. 1, except for *S. typhimurium*, the very high linear correlation coefficient ($r^2 > 0.95$) of the regression analysis indicates a strong first-order relationship

FIG. 1. Ultraviolet (UV) inactivation of microorganisms in phosphate buffered saline (PBS).

FIG. 2. UV inactivation of *S. typhimurium* with different starting populations. CFU, colony-forming units.

between the logarithm survival rate ($\log$) and the UV dose. First-order k was $0.4353 \text{ cm}^2/\text{mJ}$ for *E. coli*, $1.5614 \text{ cm}^2/\text{mJ}$ for *S. dysenteriae*, $0.10423 \text{ cm}^2/\text{mJ}$ for rotavirus and $0.0516 \text{ cm}^2/\text{mJ}$ for MS2. Of all the tested bacteria, *S. dysenteriae* was the most sensitive to UV disinfection. *S. typhimurium* was more UV resistant than *E. coli* and *S. dysenteriae*, which was inactivated $\sim 5.71 \log$ at a UV dose of $40 \text{ mJ}/\text{cm}^2$. The results show that *E. coli* cannot indicate the disinfection efficiency of *S. typhimurium* in drinking water systems. Furthermore, the dose response data indicate that rotaviruses are more resistant to UV disinfection than all the tested enteric bacteria. As shown in Fig. 1, bacteriophage T4 was more sensitive to UV irradiation than rotavirus. Of the enteroviruses and enteric bacteria used in this study, MS2 exhibited the least susceptibility to UV light, suggesting that it is a good surrogate for disinfection.

As shown in Fig. 2, at a concentration of $10^7 \text{ CFU}/\text{mL}$, *S. typhimurium* showed three distinct phases of inactivation kinetics. Initially, a shoulder is observed at a low UV dose of $4 \text{ mJ}/\text{cm}^2$. Subsequently, a first-order linear relationship is observed between the $\log$ and a UV dose between $4 \text{ mJ}/\text{cm}^2$ and $8 \text{ mJ}/\text{cm}^2$. With UV doses above $8 \text{ mJ}/\text{cm}^2$, a reduced inactivation rate was observed and tailing phenomenon occurred. The characteristic kinetic constants for the first, second, and third inactivation phases were 0.2094 , 0.9041 , and $0.0508 \text{ cm}^2/\text{mJ}$, respectively. The same inactivation response was observed when the initial population densities of *S. typhimurium* were $10^4 \text{ CFU}/\text{mL}$, but the tailing phenomenon did not occur. The results confirmed that aggregation of *S. typhimurium* suspension during UV treatment resulted in the tailing phase of the inactivation. Furthermore, no significant difference in PBS, DW, and SW water samples was observed for the inactivation of the tested bacteria, as shown Fig. 3.

Photoreactivation of enteric bacteria inactivated by UV

Since a virus does not have repair enzymes by itself (Furuta *et al.*, 1997), the photoreactivation of HRV-Wa did not occur after UV irradiation. However, the repair of bacteria under a fluorescent lamp was investigated in this study. Figure 4 illustrates the photoreactivation kinetics of bacteria after exposure to different UV doses. The photoreactivation rates of

FIG. 3. UV dose-response curves of the tested bacteria and virus in various water matrices. (A) *S. typhimurium*; (B) *S. dysenteriae*; (C) rotavirus. DW, treated drinking water; SW, local surface water.

the three UV inactivated bacteria decreased with increasing UV doses. At the beginning stage, the induction period, the UV damaged bacteria exhibited imperceptible reactivation, then rapid exponential growth. As shown in Fig. 4, for the *E. coli* inactivated by a UV dose of $8 \text{ mJ}/\text{cm}^2$, the induction period was $\sim 30 \text{ min}$. With longer times, *E. coli* repair increased rapidly, and photoreactivation reached the maximum level of 2.28% after 180 min . However, the photoreactivation levels of

FIG. 4. Photoreactivation kinetics of *E. coli* after exposure to different UV doses in DW sample.

E. coli inactivated by a UV dose of 14 mJ/cm^2 were much lower. Only 0.45 log *E. coli* were reactivated under otherwise identical conditions. The higher UV dose would seem to induce greater damage to the DNA, decreasing the repair ability of *E. coli*. Supplementary Fig. S1 shows a similar phenomenon was observed for *S. typhimurium* and *S. dysenteriae*. The results suggest that higher UV doses should be applied for completely eliminating photoreactivation when drinking water is disinfected with UV light.

As shown in Fig. 5, after a UV dose of 8 mJ/cm^2 , the photoreactivation level of *E. coli* in 3 hours was about 7.28%, 2.28%, 2.58% in PBS, DW, and SW, respectively. The level of photoreactivation in PBS was higher than that in the two water samples. It may be that there are some photosensitizers, such as humic acid, NO_3^- , in water that can be excited by light to generate reactive oxygen species, inhibiting the bacterial repair.

Inhibition of bacterial photorepair with chlorine

The effects of different chlorine concentrations on the photorepair of *S. dysenteriae* are shown in Fig. 6A. Four log of *S. dysenteriae* was inactivated by a UV dose of 4 mJ/cm^2 , and the residual *S. dysenteriae* was 3.59 log. At 120 min without the

FIG. 5. Photoreactivation kinetics of *E. coli* in different water samples after exposed to 8 mJ/cm^2 UV.

FIG. 6. Effect of chlorine dosage on the photoreactivation of enteric bacteria after UV disinfection. (A) *S. dysenteriae*. (B) *S. typhimurium*.

addition of chlorine, 2.13 log of *S. dysenteriae* were reactivated. In contrast, the photoreactivation was completely inhibited and the residual *S. dysenteriae* was almost completely inactivated at 120 min with the addition of 1 mg/L chlorine. At a chlorine concentration of 2 mg/L, the residual *S. dysenteriae* was inactivated within 5 min. As shown in Fig. 6B, a similar trend was observed for *S. typhimurium*. In the absence of chlorine, the concentration of *S. typhimurium* increased from 3.1 log to 6.5 log during photoreactivation under fluorescent light. The addition of 1 mg/L chlorine was only adequate in inactivating bacteria in 10 minutes. After 10 min, no residual chlorine was detected due to the rapid depletion of the chlorine, and the photoreactivation of *S. typhimurium* still occurred. At a dosage of 2 mg/L chlorine, photoreactivation did not occur, and *S. dysenteriae* inactivated within 10 min. These results indicated that the combination of a low dose of UV and a low concentration of chlorine can effectively inactivate bacteria and inhibit the photoreactivation of UV-damaged bacteria.

Conclusions

Viruses (HRV-Wa, MS2, and T4) were more resistant to UV disinfection than the tested enteric bacteria. The UV inactivation rate was not affected by the quality of the waters that were tested. MS2 can be used as a conservative surrogate in

UV disinfection. The UV-damaged pathogenic bacteria were significantly photoreactivated under fluorescent light irradiation. Moreover, the inactivated bacteria exhibited different photoreactivation rates with different water samples. The higher UV doses used in this study were able to inhibit photoreactivation of the bacteria. The combination of a low dose of UV and a low concentration of chlorine not only can inhibit the photoreactivation of UV-damaged bacteria but also can inactivate bacteria effectively.

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Author Disclosure Statement

No competing financial interests exist.

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