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Sequential use of ultraviolet light and chlorine for reclaimed water disinfection

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Abstract

Several disinfection processes of ultraviolet (UV), chlorine or UV followed by chlorine were investigated in municipal wastewater according to the inactivation of *Escherichia coli*, *Shigella dysenteriae* and toxicity formation. The UV inactivation of the tested pathogenic bacteria was not affected by the quality of water. It was found that the inactivated bacteria were obviously reactivated after one day in dark. Fluorescent light irradiation increased the bacteria repair. The increase of UV dosage could cause more damage to bacteria to inhibit bacteria self-repair. No photoreactivation was detected when the UV dose was up to 80 mJ/cm^2 for *E. coli* DH5α, and 23 mJ/cm^2 for *S. dysenteriae*. Nevertheless, sequential use of 8 mJ/cm^2 of UV and low concentration of chlorine (1.5 mg/L) could effectively inhibit the photoreactivation and inactivate *E. coli* below the detection limits within seven days. Compared to chlorination alone, the sequential disinfection decreased the genotoxicity of treated wastewater, especially for the sample with high NH_3-N concentration.

Key words: chlorine disinfection; sequential disinfection; UV disinfection; photoreactivation; genotoxicity; wastewater reclamation

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Introduction

Reclaimed water as an alternative water source for domestic, industrial, agricultural and recreational purposes has increasing contribution to sustainable water resources in many areas of the world. To minimize the public risks associated with exposure to reclaimed water, adequate disinfection process is necessary. Chlorination is the traditional and most common wastewater disinfection method used around the world. However, the use of chlorination has been decreasing, mainly due to toxic, mutagenic and/or carcinogenic disinfection by products (DBPs) and chlorine residuals formed in the disinfection process (Ates et al., 2007; Yang et al., 2005). Moreover, chlorination has lower efficiency against viruses, bacterial spores and protozoan cysts (Korich et al., 1990; Tyrrell et al., 1995). Therefore, the development of effective disinfection technologies has been an issue.

Ultraviolet irradiation is a promising alternative to the use of free chlorine as a means of primary disinfection due to its proven high inactivation capability against *Cryptosporidium parvum* without producing DBPs at common disinfection doses (Craik et al., 2001; Liberti et al., 2003; Liu et al., 2002). However, due to its inability to maintain residuals and the possibility for repair of UV-damaged microorganisms (Hassen et al., 2000; Oguma et al., 2004), UV irradiation could not be used in wastewater treatment as a stand-alone disinfectant.

It is current investigation that UV irradiation acts as the primary disinfection, while a secondary disinfectant, such as free chlorine or monochloramine to provide residuals to prevent subsequent microbial regrowth (Ballester and Malley, 2004; Shang et al., 2007). With the application of both physical and chemical disinfectants that attack different sites within a biological cell, synergies may occur and this may enhance the disinfection efficiency. A number of studies in the literature have reported the occurrence of synergistic effects among various disinfectant pairs. In the combined use of UV with free chlorine, prior low-pressure (LP) UV damage to *E. coli*, MS2 coliphage and PRD-1 somatic phage was reported to enhance the susceptibility of these microorganisms to subsequent free chlorine exposure (Kashinkunti et al., 2004). It was observed that the addition of 0.5 mg/L monochloramine resulted in suppression of photoreactivation for 1 hr only. An increased monochloramine dose of 1 mg/L was found to prevent photoreactivation for the entire duration of experiment (Quek et al., 2006). Therefore, the sequential disinfection of UV followed by chemical disinfectants is a potential approach for the wastewater treatment.

The major objective of this work is to investigate the wastewater disinfection by UV, chlorine or UV followed

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by chlorine in municipal wastewater samples in terms of bacteria inactivation and toxicity formation. The combination conditions for UV and chlorine disinfection processes were optimized and discussed in the disinfection of different wastewaters.

1 Experimental section

1.1 Materials and reagents

The bacteria strains used in this study are pathogenic bacterium *E. coli* DH5α and *S. dysenteriae* CGMCC 1.2428, obtained from Institute of Microbiology, Chinese Academy of Sciences. *E. coli* ATCC15597 was from American Type Culture Collection, which served as conservative indicators for photoreactivation due to its higher photo-repair (Quek and Hu, 2008). All chemicals used were analytical grade, purchased from Beijing Chemical Company and used without further purification.

Wastewater samples were collected at the position before disinfection from two wastewater reclamation plants where a membrane bioreactor (MB) (W1) and a microfilter (MF) (W2) were used as the main treatment processes, respectively. The samples were filtrated and stored at 4°C. The parameters including pH, ammonia nitrogen (Nesslerizatin method), dissolved organic carbon (Phoenix 8000, Tekmar-Dohrmann, USA), $UV_{254\,nm}$ (UV-Vis Spectrophotometer-U-3100, Hitachi Co., Japan) were determined before the experiments and the results are shown in Table 1.

Table 1 Water quality characteristics of wastewater samples used in this study

Sample	Source	pH	$C_{NH_3\text{-}N}$ (mg/L)	C_{DOC} (mg/L)	UV_{254} (cm^{-1})
W1	MB	8.0	0.25	7.47	0.193
W2	MF	8.3	6.98	5.51	0.108

MB: membrane bioreactor; MF: microfilter.
W1: treated with MB; W2: treated with MF.

1.2 Preparation and analysis of microorganism

The stock suspension of *E. coli* and *S. dysenteriae* were prepared following the procedure described elsewhere in the literature (Hu et al., 2007). For each strain, 1 mL of the frozen stock culture was inoculated into 50 mL of Luria-Bertani nutrient solution (LB) and incubated at 37°C for 18 hr with shaking at 130 r/min. Then 0.2 mL of this culture was added to 50 mL of fresh LB and incubated for 18 hr at 37°C to obtain stationary phase culture. The cells were harvested by centrifuging at 3000 r/min for 10 min, washed three times with phosphate buffered saline (PBS, 0.01 mol/L, pH 7.2). Then the cells were resuspended and diluted to ca. 1×10^8 colony forming units (CFU/mL) with PBS or wastewater and used immediately for disinfection procedure. The bacteria concentration of *S. dysenteriae* and *E. coli* was determined by the spread plate method on nutrient agar after growing for 48 hr at 37°C.

1.3 UV disinfection and photoreactivation

A low-pressure mercury lamp (11 W, Beijing Lighting Research Institute, China) was housed in a collimating apparatus and used as the source of UV radiation. Through the collimating tube, a quasi parallel beam was focused onto a Petri dish filled with 20 mL test solution, which was magnetically stirred throughout experiments. At the center of solution surface, the incident intensity was approximately 0.125 mW/cm^2. The average UV intensity was calculated as described by Bolton and Linden (2003). Required exposure times were determined by the desired UV dose according to the average UV intensity.

The photoreactivation experiments were performed under three fluorescent lamps, wherein the fluorescent light intensity at 360 nm was 0.066 mW/cm^2. The percentage of photoreactivation was calculated as follows (Lindenauer and Darby, 1994):

$$\text{Percentage of photoreactivation} = \frac{\text{Number of cells photoreactivated}}{\text{Number of cells inactivated by UV}} \times 100\%$$

1.4 Chlorine disinfection

Sodium hypochlorite of 9% was diluted in ultrapure water to prepare stock solution. Chlorine concentration was measured by N,N-diethyl-p-phenylenediamine (DPD) colorimetric method according to the standard method of Water and Wastewater Monitoring of China. Chlorine residual in samples for bacteria enumeration was immediately neutralized with 1% sodium thiosulfate. All experiments were repeated three times and were performed at room temperature (25 ± 2°C). All materials used in the experiments were autoclaved at 121°C for 25 min to ensure sterility.

1.5 Umu test

Wastewater samples before and after disinfection treatments were concentrated by the same method described in literature (Wang et al., 2006). The umu tests for genotoxicity of different samples were performed with *Salmonella typhimurium* TA1535/pSK1002 without S9 activation according to ISO 13829. In this assay, 4-nitroquinoline oxide was used as the positive reference and dimethyl sulfoxide as the negative control. When the induction ratio (IR) value is over 2.0, the test results were judged positive.

2 Results and discussion

2.1 UV disinfection

Figure 1 shows the inactivation kinetics of the three tested bacteria by different UV doses in PBS and two wastewater samples (W1 and W2) at room temperature. As shown in Fig. 1a, the inactivation curve of *E. coli* DH5α was characterized mainly by two phases, an initial phase with 4.5–4.9 log reduction of *E. coli* DH5α and a tailing phase at higher UV doses (> 2 mJ/cm^2) corresponding to a lower inactivation rate. In the tailing, the lower rate was contributed to UV-resistant bacteria, or bacteria aggregated

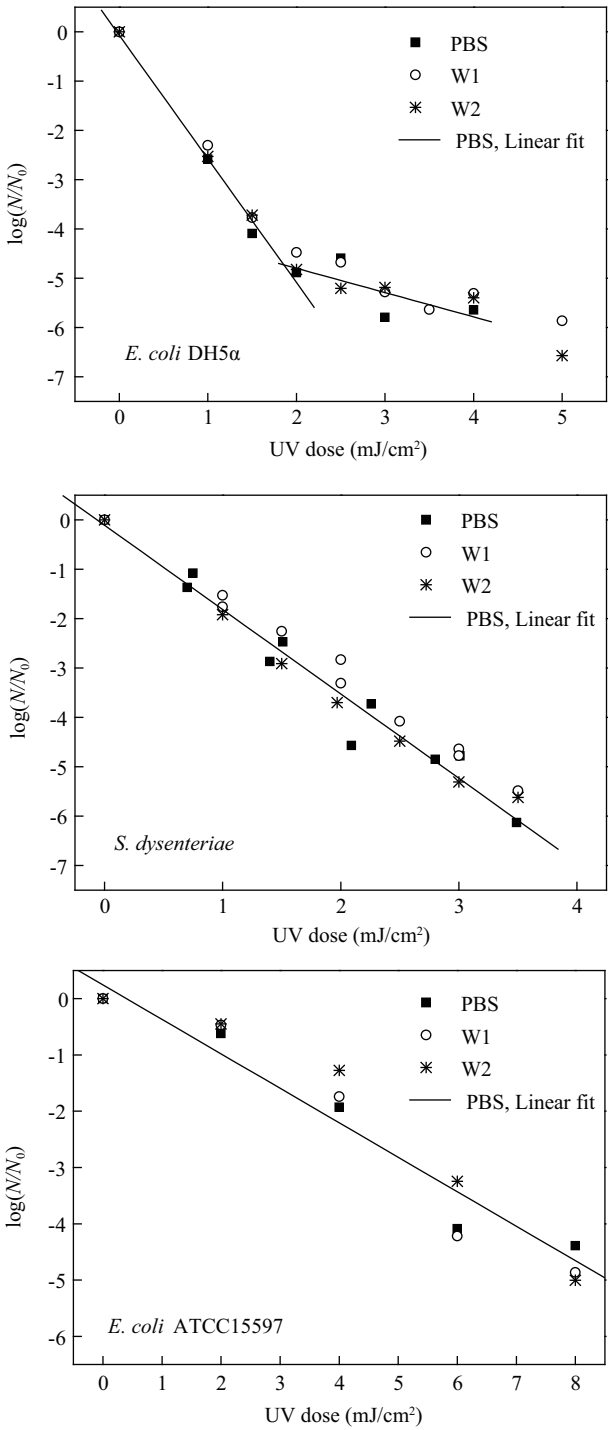

Fig. 1 UV dose-response curves of the tested bacteria in the three water matrices. W1: the wastewater treated with membrance bioreactor; W2: the wastewater treated with microfilter; PBS: phosphate buffered saline.

in small groups in which some UV protection is provided by peripheral bacteria. The inactivation of *S. dysenteriae* and *E. coli* ATCC15597 agreed well with the first-order kinetics model. The inactivation rate of *S. dysenteriae* and *E. coli* ATCC15597 in PBS was 1.708 and 0.612, respectively, which is comparable with the UV-sensitivity of bacteria reported in literature (Hijnen et al., 2006). The quality of wastewater did not greatly affect the inactivation kinetics of the three tested organisms.

Furthermore, the reactivation of the inactivated bacteria was examined in dark or under light irradiation. The

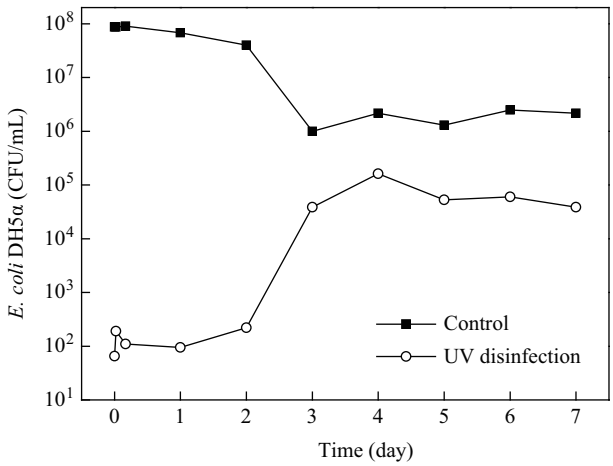

Fig. 2 *E. coli* DH5α concentration versus incubation time in dark after pretreatment by UV dose of 8 mJ/cm^2 and non-irradiation in W1 sample.

inactivated *E. coli* DH5α by UV dose of 8 mJ/cm^2 kept in dark at room temperature are shown in Fig. 2. No significant *E. coli* DH5α increasing was observed within 8 hr of incubation. However, after 1 day, the *E. coli* DH5α was greatly reactivated, and 10^5 CFU/mL *E. coli* DH5α were determined in 4th day. Under the identical condition, the untreated *E. coli* DH5α at 10^8 CFU/mL did not significantly propagate. The results indicated the UV-damaged bacteria had dark-repair ability after long incubation time.

Under fluorescent light irradiation, the repair of *E. coli* ATCC15597 repair was more obvious and reached the maximum level after 2 hr as shown in Fig. 3. In PBS, W1 and W2, the maximum level of photoreactivation was about 7.94%, 0.80% and 2.93%, respectively. The level of photoreactivation in PBS was higher than that in the two wastewater samples. It may be that there are some photosensitizers such as humic acid, NO_3^- in wastewater, which could be excited by light to generate reactive oxygen species, inhibiting the bacteria repair (Liltved and Landfald, 2000).

Figure 4 shows that the percentage photoreactivation decreased with the UV dose increasing, which was ob-

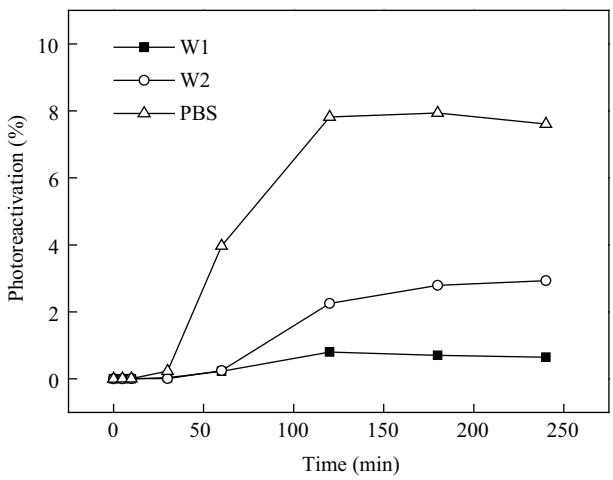

Fig. 3 Photoreactivation of *E. coli* ATCC15597 after pretreatment with 8 mJ/cm^2 UV in different water samples (the fluorescent light intensity at 360 nm was 0.066 mW/cm^2).

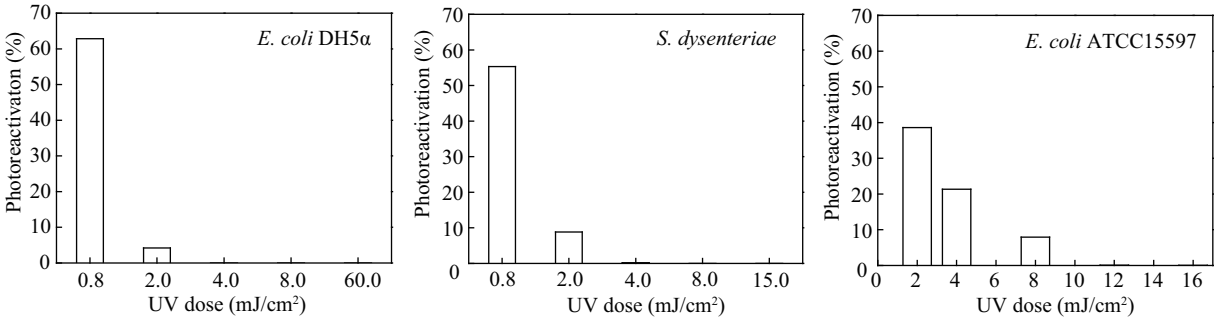

Fig. 4 Effect of UV dose on photoreactivation levels of UV-damaged bacteria in PBS.

served in many research (Lindenauer and Darby, 1994). The highest photoreactivation level of *E. coli* DH5α and *S. dysenteriae* were 62.84% and 55.32%, respectively at a UV dose of 0.8 mJ/cm^2. With the increasing of UV dose, percentage photoreactivation decreased to less than 0.10% at UV dose of 4 mJ/cm^2 and above. For *E. coli* DH5α, obvious photoreactivation was still found until 60 mJ/cm^2 UV dose applied. The photoreactivation of *S. dysenteriae* was undetectable at UV dose of 23 mJ/cm^2. For *E. coli* ATCC 15597, the percentage photoreactivation was 38.6% after UV treatment of 2 mJ/cm^2, and it decreased to less than 0.1% at the UV dose of 12 mJ/cm^2. These results illustrated that the increase of the UV dose caused more damage to bacteria to inhibit bacteria self-repair. However, because of the lack of bacteriosatic effect and possibility about photoreactivation or dark repair of UV-damaged bacteria, UV should be combined with other methods of disinfection such as chlorination to enhance the disinfection efficiency.

2.2 Chlorine disinfection

In chlorine disinfection, the indicator organism *E. coli* ATCC15597 was chosen as target microorganism and contact time of 30 min which was generally adopted in wastewater treatment plants was chosen. As shown in Fig. 5, the water quality had great influence on the chlorine disinfection. For the 4 log(*E. coli* ATCC15597 inactivation), the chlorine dose was about 1, 1.5 and 3 mg/L respectively in PBS, W1 and W2. A nearly complete inactivation of *E. coli* ATCC15597 could be achieved at a chlorine concentration of 5 mg/L and the concentration of total residual chlorine after contacting 30 min was 4.38 mg/L in W2 water. Higher efficiency of *E. coli* ATCC15597 inactivation was achieved in sample W1 than that in sample W2. This may be due to higher NH_3-N concentration in sample W2 than that in sample W1. Ammonia reacted with free chlorine to form various chloramine species (mono-, di- and tri-chloramines), which is much less bactericidal than free chlorine (Shang et al., 2005).

2.3 UV/chlorine disinfection

In sequential disinfection experiments, *E. coli* ATCC15597 was chosen as target microorganism. In this experiment, the chlorine disinfection in PBS was carried out under fluorescent light after pretreatment with UV. As shown in Fig. 6a, after pretreatment of 4 mJ/cm^2 UV, the *E. coli* ATCC15597 photoreactivation occurred without or with the addition of 0.25 mg/L initial chlorine. However, at the initial chlorine concentration 0.5 mg/L, the photoreactivation was depressed to a large extent. At the chlorine concentration 1 mg/L, not only the photoreactivation was inhibited but the bacteria was further inactivated to the undetected level. As shown in Fig. 6b, increasing the UV dose to 8 mJ/cm^2, the complete suppression of photoreactivation was achieved by the addition of 0.5 mg/L chlorine. The results indicated that the more UV damage to bacteria benefited for the chlorine disinfection.

The *E. coli* ATCC15597 after UV irradiation of 8 mJ/cm^2 was further treated in W1 and W2 with chlorine 1.5 mg/L in Fig. 7. It was found that the photoreactivation did not occur and the *E. coli* was further inactivated to below detection limits at 60 min. The total residual chlorine slowly decreased, and maintained 0.92 and 1.15 mg/L at 60 min in W1 and W2, respectively. Prolonging the contact time to 7 day, no *E. coli* ATCC15597 was detectable. Compared with the chlorine disinfection, the sequential disinfection of UV and chlorine effectively controlled photoreactivation and minimized the concentration of chlorine.

2.4 Genotoxicity of byproduct from disinfection

After disinfection procedures of chlorine disinfection (5 mg/L) and UV combined with chlorine disinfection (8 mJ/cm^2 of UV followed by 1.5 mg/L chlorine), the genotoxicity of byproducts was determined by umu/SOS

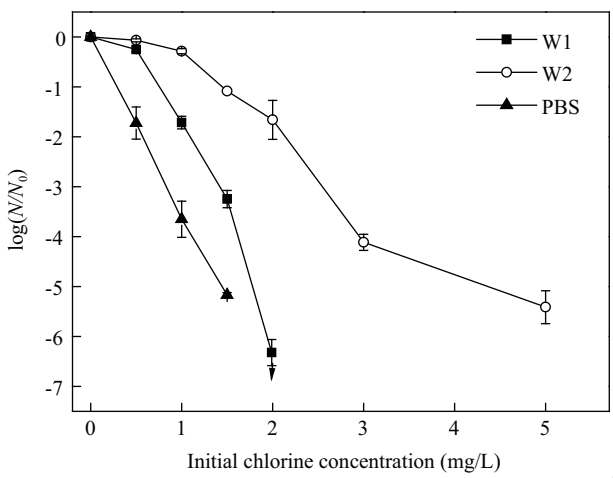

Fig. 5 Effect of water quality on the inactivation of *E. coli* ATCC15597 by chlorine after contact time of 30 min.

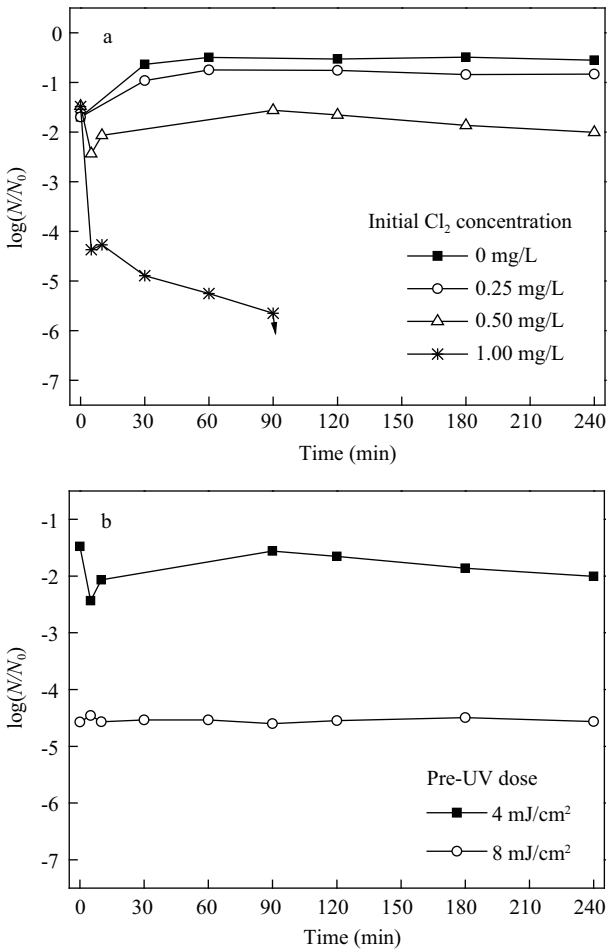

Fig. 6 Inactivation curve of *E. coli* ATCC15597 with different levels of chlorine under fluorescent light after pretreatment with UV of 4 mJ/cm^2 (a) and curve with 0.5 mg/L chlorine under fluorescent light after different levels of UV pretreatment (b) in PBS.

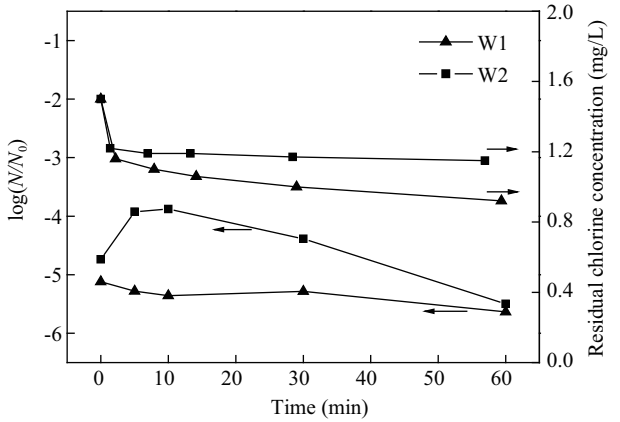

Fig. 7 Inactivation of *E. coli* and chlorine consuming in two filtered wastewater samples with 1.5 mg/L of chlorine after pretreatment with UV of 8 mJ/cm^2.

in Fig. 8. After both disinfection procedures, W1 samples exhibited similar genotoxicity to the raw sample. However, in the W2, introduction ratio values of raw sample and the sample treated by UV and chlorine disinfection were less than 2, indicating its negative genotoxicity, while that one of the sample treated by chlorination were much more than 2, indicating its positive genotoxicity. Therefore, the combination of UV/chlorine comparing to chlorination alone could reduce the concentration of chlorine, leading to decrease the genotoxicity of effluent in W2. The NH_3-N concentration of samples W2 was 6.98 mg/L, much higher than that of sample W1 (0.25 mg/L), as shown in Table 1. The higher genotoxicity in W2 was possibly due to the higher NH_3-N, which is agreed with the work reported by Wang et al. (2006). Their investigation indicated that the genotoxicity of chlorinated wastewater increased obviously with the increasing of NH_3-N concentration. Chlorination disinfection of wastewater might lead harmful influence to the ecological system in the presence of the higher NH_3-N concentration. The genotoxicity of effluent could be controlled by sequential use of UV and chlorine.

3 Conclusions

The UV inactivation rate of the tested pathogenic bacteria was not affected by the quality of water. However, the UV-damaged bacteria were greatly reactivated after 1 day in dark. Fluorescent light irradiation increased the bacteria repair. Moreover, the inactivated bacteria exhibited different photoreactivation rates under different water quality. The increase of the UV dose could cause more damage

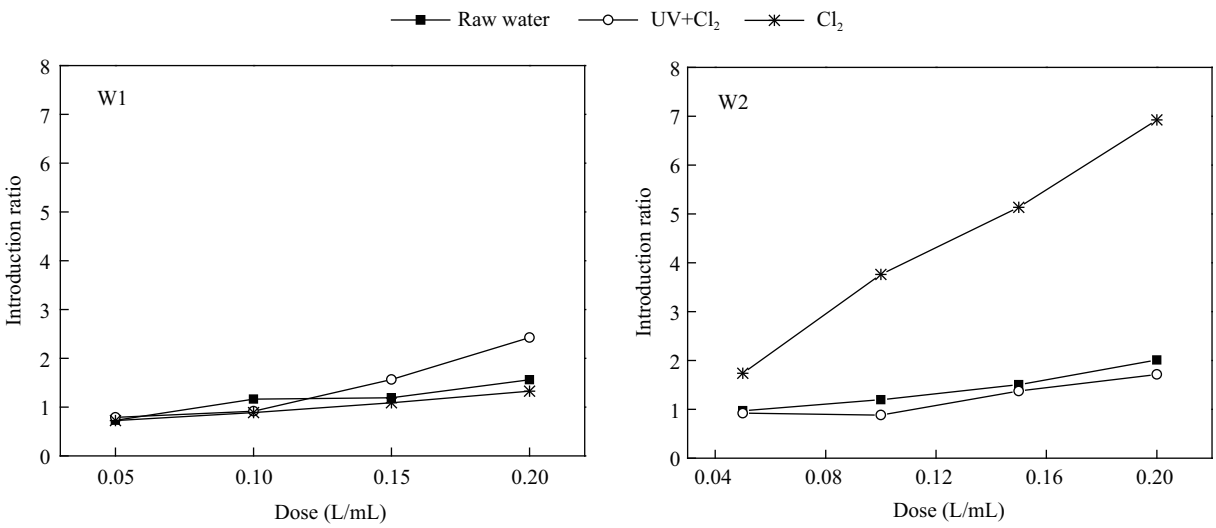

Fig. 8 Genotoxicity changes during different disinfection procedure. UV+Cl_2: 8 mJ/cm^2 UV irradiation followed by 1.5 mg/L chlorine; Cl_2: 5 mg/L.

to bacteria to inhibit bacteria self-repair. The sequential disinfection of UV and chlorine could effectively control photoreactivation and minimize the concentration of chlorine, decrease the genotoxicity of treated wastewater.

Acknowledgments

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References

Ates N, Kitis M, Yetis U, 2007. Formation of chlorination by-products in waters with low SUVA–correlations with SUVA and differential UV spectroscopy. *Water Research*, 41(18): 4139–4148.

Ballester N A, MAlley J P Jr, 2004. Sequential disinfection of adenovirus type 2 with UV-chlorine-chloramine. *Journal of the American Water Works Association*, 96(10): 97–103.

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

Craik S A, Weldon D, Finch G R, Bolton J R, Belosevic M, 2001. Inactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. *Water Research*, 35(6): 1387–1398.

Hassen A, Mahrouk M, Ouzari H, Cherif M, Boudabous A, Damelincourt J J, 2000. UV disinfection of treated wastewater in a large-scale pilot plant and inactivation of selected bacteria in a laboratory UV device. *Bioresource Technology*, 74(2): 141–150.

Hijnen W A M, 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.

Hu C, Guo J, Qu J H, Hu X X, 2007. Photocatalytic degradation of pathogenic bacteria with AgI/TiO_2 under visible light irradiation. *Langmuir*, 23(9): 4982–4987.

Kashinkunti R D, Linden K G, Shin G A, Metz D H, Sobsey M D, Moran M C et al., 2004. Investigating multibarrier inactivation for Cincinnati: UV, by-products, and biostability. *Journal of the American Water Works Association*, 96(6): 114–127.

Korich D G, Mead J R, Madore M S, Sinclair N A, Sterling C R, 1990. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Applied Environmental Microbiology*, 56(5): 1423–1428.

Liberti L, Notarnicola M, Petruzzelli D, 2003. Advanced treatment for municipal wastewater reuse in agriculture. UV disinfection: parasite removal and by-product formation. *Desalination*, 152(1-3): 315–324.

Liltved H, Landfald B, 2000. Effects of high intensity light on ultraviolet-irradiated and non-irradiated fish pathogenic bacteria. *Water Research*, 34(2): 481–486.

Lindenauer K G, Darby J L, 1994. Ultraviolet disinfection of wastewater: Effect of dose on subsequent photoreactivation. *Water Research*, 28(4): 805–817.

Liu W J, Andrews S A, Bolton J R, Linden K G, Sharpless C M, Stefan M, 2002. Comparison of DBP formation from different UV technologies at bench scale. *Water Science and Technology: Water Supply*, 2(5-6): 515–521.

Oguma K, Katayama H, Ohgaki S, 2004. Photoreactivation of *Legionella pneumophila* after inactivation by low- or medium-pressure ultraviolet lamp. *Water Research*, 38(11): 2757–2763.

Quek P H, Hu J Y, Chu X N, Feng Y Y, Tan X L, 2006. Photoreactivation of *Escherichia coli* following medium-pressure ultraviolet disinfection and its control using chloramination. *Water Science and Technology*, 53(6): 123–129.

Quek P H, Hu J Y, 2008. Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiology and Biotechnology*, 35(6): 533–541.

Shang C, Cheung L M, Liu W, 2007. MS2 coliphage inactivation with UV irradiation and free chlorine/monochloramine. *Environmental Engineering Science*, 24(9): 1321–1332.

Shang C, Qi Y N, Lo I M C, 2005. Factors affecting inactivation behavior in the monochloramination range. *Journal of Environmental Engineering*, 131(1): 119–129.

Tyrrell S A, Rippey S R, Watkins W D, 1995. Inactivation of bacterial and viral indicators in secondary sewage effluents, using chlorine and ozone. *Water Research*, 29(11): 2483–2490.

Wang L S, Hu H Y, Wang C, 2006. Effect of ammonia nitrogen and dissolved organic Matter fractions on the genotoxicity of wastewater effluent during chlorine disinfection. *Environmental Science & Technology*, 41(1): 160–165.

Yang X, Shang C, Huang J C, 2005. DBP formation in breakpoint chlorination of wastewater. *Water Research*, 39(19): 4755–4767.