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UV light tolerance and reactivation potential of tetracycline-resistant bacteria from secondary effluents of a wastewater treatment plant

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ABSTRACT

Tetracycline-resistant bacteria (TRB) are of concern as emerging microbial contaminants in reclaimed water. To understand the effects of UV disinfection on TRB, both inactivation and reactivation profiles of TRB, as well as 16 tetracycline-resistant isolates from secondary effluent, were characterized in this study. The inactivation ratio of TRB was significantly lower (3.0-log) than that of heterotrophic bacteria (>4.0-log) in the secondary effluent. Additionally, the proportion of TRB significantly increased from 1.65% to 15.51% under 20 mJ/cm^2 ultraviolet (UV) exposure. The inactivation rates of tetracycline-resistant isolates ranged from 0.57/s to 1.04/s, of which tetracycline-resistant Enterobacter-1 was the most tolerant to UV light. The reactivation of TRB, tetracycline-resistant isolated strains, as well as heterotrophic bacteria commonly occurred in the secondary effluent even after 20 mJ/cm^2 UV exposure. The colony forming ability of TRB and heterotrophic bacteria reached 3.2-log and 3.0-log under 20 mJ/cm^2 UV exposure after 22 hr incubation. The final inactivation ratio of tetracycline-resistant Enterobacter-1 was 1.18-log under 20 mJ/cm^2 UV exposure after 22 hr incubation, which is similar to those of TRB (1.18-log) and heterotrophic bacteria (1.19-log). The increased proportion of TRB and the reactivation of tetracycline-resistant enterobacteria in reclaimed water could induce a microbial health risk during wastewater reuse.

Introduction

Wastewater reclamation and reuse reduces pressure on water resources for municipal usage, landscaping, recreation areas, *etc.*, making it an important part of the total water cycle in cities (Yang and Abbaspour, 2007). During reclaimed water recycling, waterborne pathogens, which cause infectious disease, are the main concern for public health (Hunter, 2002; Amahmid and Bouhoum, 2005; Toze, 2006; World Health Organization (WHO), 2006; Campos, 2008).

A disinfection procedure during the process of reclaimed water production is necessary for pathogen control. Among

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common disinfection processes, ultraviolet (UV) disinfection has excellent inactivation for a majority of viruses, bacteria, and protozoa, with the advantage of short contact times (Hijnen et al., 2006), as well as formation of few disinfection by-products (Nurizzo et al., 2005). All in all, UV disinfection is considered a safe, effective and cost-competitive option for pathogen control in wastewater, thus, ensuring public health safety during reclaimed water recycling (Rusin and Gerba, 2001).

In addition to pathogens, antibiotic-resistant bacteria (ARB) are emerging contaminants in wastewater (Pruden, et al., 2006; Sapkota, et al., 2007; Li, et al., 2009). Some studies have showed that ARB in the effluent from municipal wastewater treatment plants (WWTP) discharged into natural water may induce the spread of ARB in the environment (Goñi-Urriza et al., 2000; LaPara et al., 2011). ARB are not considered primary pathogens; however, their ability to transmit resistance to other bacteria (including pathogens) through transmissible resistance factors is an important concern (Séveno, et al., 2002; Bennett 2008; Martínez, 2008). Due to the wide use of tetracycline antibiotics, TRB and tetracycline resistance genes are prevalent in the environment (McKeon, et al., 1995; Macauley et al., 2007; Peak et al., 2007; Sapkota et al., 2007; Baquero, et al., 2008). Research shows that WWTP effluents could be a major source of TRB in surface water (Zhang, et al., 2009).

There is inadequate data regarding the effects of UV disinfection on the proportion or UV light tolerance of TRB in reclaimed water. Meckes showed that the percentage of total coliforms resistant to tetracycline in the waste water effluent after disinfection by UV light was significantly higher than before UV disinfection (Meckes, 1982). Staley et al. (1988) also conducted a series of experiments and found that there was an increase in fecal coliforms resistant to tetracycline after UV disinfection. Guo et al. (2013a, 2013b) also showed that UV disinfection led to enrichment of bacteria resistant to tetracycline, and the proportion of TRB was nearly double that before UV disinfection. McKinney and Pruden found that two Gram-positive antibiotic-resistant bacteria (methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*) were more resistant to UV disinfection than the two Gram-negative antibiotic-resistant bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) studied (McKinney and Pruden, 2012). Regardless, research on the UV disinfection rate of TRB with different genotypes is still limited.

Although reactivation is common among enteric bacteria after UV disinfection in reclaimed water (Lazarova et al., 1999; Hijnen et al., 2006; Guo et al., 2009), there are few studies on the dark repair of ARB in reclaimed water after UV disinfection.

The main objective of this study was to explore and characterize the effect that UV disinfection has on TRB, and to assist in estimating the retention and reappearance of TRB in reclaimed water after UV disinfection. In order to judge the viability and recovery of TRB, the inactivation and dark repair potential of heterotrophic bacteria and TRB in the secondary effluent were compared under a series of UV doses. Also, the inactivation and dark repair potential of 16 tetracycline-resistant strains isolated from the secondary effluent were analyzed to indicate any genus of TRB expressing tolerance to UV light or high reactivation potential after disinfection.

1. Materials and methods

1.1. Water samples and microorganisms

The reclaimed water used in the experiments was collected from the secondary sedimentation tank of a municipal wastewater treatment plant in Beijing, China. The treatment processes of this plant include primary sedimentation, anaerobic-anoxic-oxic treatment, and secondary sedimentation processes. All samples were axenically collected in sterile containers and transported to the lab on ice for immediate processing. The concentrations of chemical oxygen demand (COD), total organic carbon (TOC), and ammonia nitrogen of the three water samples were 82.0–111.0, 9.0–15.9, and 8.8 mg/L, respectively. The pH of the water samples was 8.0–8.1. The absorbance at 254 nm (UV_{254}) of the water samples was 0.14–0.21. The concentration of heterotrophic bacteria in the secondary effluent was 1.1×10^5–1.9×10^5 CFU/mL.

Water samples containing tetracycline-resistant isolates were prepared as follows: culture in nutrient broth (peptone: 10.0 g/L, beef extract: 3.0 g/L, NaCl: 5.0 g/L, pH = 7.2) with 16 mg/L tetracycline at 37°C in an airbath incubator with agitation at 110–120 r/min until the end of logarithmic phase was reached. All stock solutions of tetracycline were prepared at a concentration of 800 mg/L dissolved in 70% (V/V) ethanol solution and stored at −20°C for up to one month. The cells were collected and centrifugation was performed at 10,000 r/min, 10 min, 4°C. Cells were then washed with sterile phosphate-buffered saline (PBS, pH = 7.4) twice, and subsequently suspended in the secondary effluent (filtered by 0.22 μm filters), achieving an initial concentration of approximately 10^7 CFU/mL.

Nutrient agar plates with 16 mg/L tetracycline were used to screen for TRB from the WWTP's secondary sedimentation unit on April 17th, 2010. Representative tetracycline-resistant isolates were purified on nutrient agar plates with 16 mg/L tetracycline. The isolates were identified by 16S ribosomal DNA sequencing (by Takara Bio Inc.) combined with BLAST searching (NCBI, National Center of Biotechnology Information).

The end of the logarithmic phase of the growth curve of the tetracycline-resistant isolate was determined prior to UV disinfection. A single colony was inoculated into nutrient broth with 16 mg/L tetracycline and incubated in an airbath at 37°C, 110–120 r/min. OD_{600} was measured every 2 hr to determine the growth curves of the isolates.

1.2. Laboratory UV disinfection and dark repair experiments

Water samples (15 mL) were transferred into 60-mm Petri dishes, put under a collimated tube, gently mixed with a magnetic stirbar, and exposed to UV light at room temperature (25 ± 2°C) for the designated time. The collimated UV light was supplied by a low-pressure UV light source (253.7 nm). The irradiance value was determined by a radiometer before UV irradiation and remained stable throughout the experiments. The average irradiance value and exposure time at a fixed UV dose on the water surface was calculated by the method described by Bolton and Linden (2003).

Repair experiments were carried out after UV exposure in the dark to simulate conditions of reclaimed water stored in pipelines and in a tank. The unexposed water samples and exposed water samples were allowed to stand in the original Petri dishes at room temperature (25 ± 2°C) for 22 hr. All disinfection and repair experiments were replicated in duplicate.

1.3. Microbial analysis

Heterotrophic bacteria (ISO, 6222, 1999) were enumerated by diluting 1 mL of sample into 10 mL nutrient agar (peptone: 10 g/L, beef extract: 3 g/L, NaCl: 5 g/L, agar: 15 g/L, pH = 7.2). To obtain colony counts between 30 and 300 per plate, all water samples were diluted by ten-fold serial dilutions in PBS. Plates were replicated in triplicate and incubated at 37°C for 24 hr.

TRB were also enumerated by diluting 1 mL of sample into 10 mL nutrient agar containing 16 mg/L tetracycline. Likewise, water samples were diluted using PBS to obtain 30–300 colonies of TRB per plate, and plates were incubated at 37°C for 24 hr. The concentration of tetracycline was defined as the maximum value of all minimum inhibitory concentrations (MICs) for pathogens listed in CLSI (Clinical and Laboratory Standards Institute) documentation (Clinical Laboratory Standards Institute (CLSI), 2006). The plates were replicated in triplicate.

1.4. Quantitative evaluation of inactivation of TRB

To evaluate the effects of UV irradiation on TRB, the degree of inactivation was quantified using plate counts as follows. Eq. (1) shows the log ratio of colony forming units (CFU) after inactivation to CFU before UV disinfection:

$$\text{Inactivation} = \log\left(\frac{N_0}{N_i}\right) \quad (1)$$

where bacteria included heterotrophic bacteria and TRB in the secondary effluent as well as tetracycline-resistant isolates from the secondary effluent. N_0 (CFU/mL) is plate count before UV disinfection, and N_i (CFU/mL) is immediate survival after UV disinfection.

To evaluate the change of TRB in the microbial community of the secondary effluent after UV disinfection, the percentage of TRB was quantified as follows.

$$\text{TRB}\ (\%) = \frac{N_i}{N_i^T} \times 100\% \quad (2)$$

where N_i(CFU/mL) is immediate survival of TRB in the secondary effluent after UV disinfection, and N_i^T (CFU/mL) is immediate survival of heterotrophic bacteria after the same UV dose exposure, i = 0, when the dose of UV disinfection was 0.

Inactivation kinetics of the tetracycline-resistant isolates were calculated to obtain the parameters of the inactivation kinetics, which were used to compare the tolerance of tetracycline-resistant isolates to UV light (Hijnen et al., 2006)

$$\log\left(\frac{N_0}{N_i}\right) = k \times \text{dose}_{\text{UV}} \quad (3)$$

where k (cm^2/mJ) is the inactivation rate.

The calculated UV doses varied from 2 to 10 mJ/cm^2. Tailing after 10 mJ/cm^2 was excluded in the inactivation rate calculation in this study.

1.5. Quantitative evaluation of dark repair

To evaluate dark repair after UV disinfection, the colony forming ability (CFA) $\log(\frac{N_r}{N_i})$ was used to compare the dark repair of bacteria with different initial concentrations in the secondary effluent and estimate the reactivation potential based on the final colonies of disinfected water. The colony forming ability was qualified as follows:

$$\log\left(\frac{N_r}{N_i}\right) = \log\left(\frac{N_r}{N_0}\right) + \log\left(\frac{N_0}{N_i}\right) \quad (4)$$

where N_r (CFU/mL) is plate count of the reactivated bacteria after 22 hT incubation in the dark, N_i (CFU/mL) is immediate survival after UV disinfection, and N_0 (CFU/mL) is plate count before UV disinfection.

The final inactivation after 22 h incubation in the dark was expressed as $\log(\frac{N_0}{N_r})$. Here, the final inactivation was qualified as:

$$\log\left(\frac{N_0}{N_r}\right) = \log\left(\frac{N_0}{N_i}\right) - \log\left(\frac{N_r}{N_i}\right).$$

1.6. Statistical Analysis

One-way analysis of variance (ANOVA) calculated by Origin 8.0 was used to compare the statistically significant difference of inactivation or reactivation between TRB different UV doses, and significant reactivation of tetracycline-resistant isolates after 22 hr incubation. Tukey's test, performed by Origin 8.0, compared differences of inactivation between tetracycline-resistant isolates in the secondary effluent.

2. Results and discussion

2.1. Inactivation of TRB in the secondary effluent

The secondary effluent water samples were irradiated by UV light at bench scale. The initial concentrations of heterotrophic bacteria and TRB in the secondary effluents were (1.1–1.9) × 10^5 and (2.3–2.8) × 10^3 CFU/mL, respectively.

Inactivation curves of TRB and heterotrophic bacteria are shown in Fig. 1. The results showed that the inactivation ratios of TRB were lower than these of heterotrophic bacteria at each UV dose, respectively. Remarkably, the inactivation ratios of TRB were significantly lower than these of heterotrophic bacteria when exposed to 2 and 20 mJ/cm^2 UV light, respectively (One-way ANOVA, $p < 0.05$). The inactivation of TRB was about 3.0-log with 20 mJ/cm^2 UV exposure, while that of heterotrophic bacteria was higher than 4.0-log. The result indicates that TRB survive in the secondary effluent, when the residual concentration of heterotrophic bacteria was less than 15 CFU/mL. The inactivation efficiency of TRB in the secondary effluent was lower than that of heterotrophic bacteria, which indicates that tetracycline-resistant bacteria are more tolerant to UV light than heterotrophic bacteria in the secondary effluent.

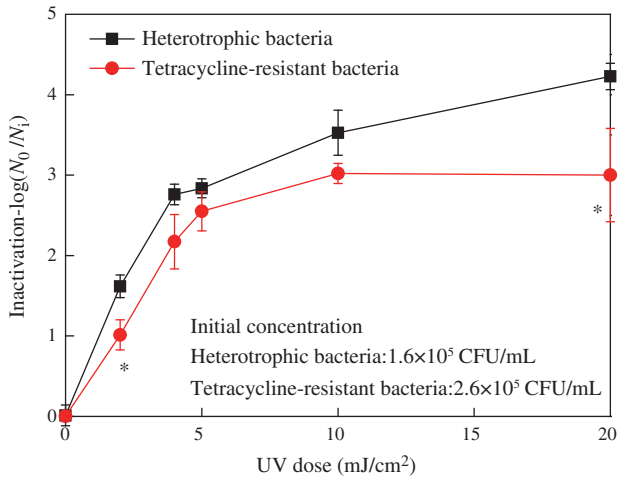

Fig. 1 – Inactivation of tetracycline-resistant bacteria and heterotrophic bacteria in the secondary effluent. Asterisk means that the difference of inactivation between heterotrophic bacteria and tetracycline-resistant bacteria is significant (One-way ANOVA, $p < 0.05$). ANOVA: analysis of variance.

However, the proportions of TRB in the secondary effluent changed after UV disinfection as follows: UV dose: 0, 2, 5, 5, 10, and 20 mJ/cm^2, corresponding changes of TRB are: (1.65 ± 0.25)%, (6.84 ± 3.18)%, (7.14 ± 5.05)%. (3.43 ± 1.71)%, (3.50 ± 2.40)%, (15.52 ± 7.31)%, respectively. Compared to the inactivation ratios of TRB, the proportions of TRB varied more widely. The proportions of TRB in the secondary effluent after UV disinfection were higher than before UV disinfection, and significantly increased after UV disinfection, from 1.65% to 15.52% after UV exposure of 20 mJ/cm^2 (One-way ANOVA, $p < 0.05$), which suggests UV disinfection selected for TRB. The work by Meckes also showed that there was a significant increase in the percentage of the surviving total coliform population from the effluent of the activated sludge process resistant to tetracycline after UV irradiation (Meckes, 1982), which is evidence to support the selection for TRB by UV disinfection in wastewater effluent. However, there is no adequate research on the internal correlation of bacteria resistant to tetracycline and tolerant to UV light.

2.2. Reactivation (dark repair) of TRB in the secondary effluent

The UV-irradiated bacteria have the ability to repair damaged DNA under either light or dark conditions, which restores the bacteria to full activity (Rusin and Gerba, 2001). Theoretically, light or dark DNA repair mechanisms could be responsible for the DNA repair of these tetracycline-resistant isolates after UV exposure (Dodd, 2014). Most likely, the occurrence of dark repair is more frequent than light repair, since the water is in the dark during reclaimed water storage and delivery. Hence, we studied the dark repair potential as the reactivation potential of TRB in the secondary effluent post UV disinfection.

The data in Fig. 2 show that the CFA of TRB was as high as that of heterotrophic bacteria after 22 h incubation. The CFA of TRB and heterotrophic bacteria reached 3.2-log and 3.0-log under 20 mJ/cm^2 UV exposure, respectively. However, the CFA of heterotrophic bacteria was significantly lower than the inactivation ratio of heterotrophic bacteria for 20 mJ/cm^2 UV exposure. The results showed that TRB can increase back to 356 CFU/mL after 22 h dark repair in the secondary effluent, which was higher than that of TRB (267 CFU/mL) after 2 mJ/cm^2 UV exposure. A previous study reported that no significant regrowth occurred in TRB or heterotrophic bacteria in the secondary effluent after 22 h incubation before UV disinfection at room temperature (Huang et al., 2013). Hence, the value of CFA here should be considered as the reactivation potential of bacteria.

2.3. Inactivation of tetracycline-resistant isolates from the secondary effluent

Since TRB in the secondary effluent showed lower inactivation by UV disinfection than that of heterotrophic bacteria, as well as exhibiting reactivation after disinfection, the inactivation and reactivation of tetracycline-resistant strains isolated from the secondary effluent were studied to see any strain expressing tolerance to UV light or reactivation potential. Sixteen randomly isolated tetracycline-resistant strains were identified by 16S rDNA sequencing combined with BLAST searching. There were five Escherichia strains, four Aeromonas strains, three Enterobacter strains, two Klebsiella strains, one Citrobacter strain, and one strain of Hafnia (Xi et al., 2014).

Since many organizations have issued guidelines and recommendations regarding UV disinfection of drinking water for the control of waterborne pathogens, the UV doses used in this study were in the range of 0 to 40 mJ/cm^2 (Toze, 2006). The inactivation ratio of tetracycline-resistant strains exposed to 40 mJ/cm^2 UV light, which yields the highest inactivation ratio, was identified as the maximum inactivation ratio. The UV dose at which inactivation ratios reached stability (UVD_S) could be considered as the minimum UV dose needed to inactivate a tetracycline-resistant strain effectively. The purpose of calculating the inactivation rate of tetracycline-

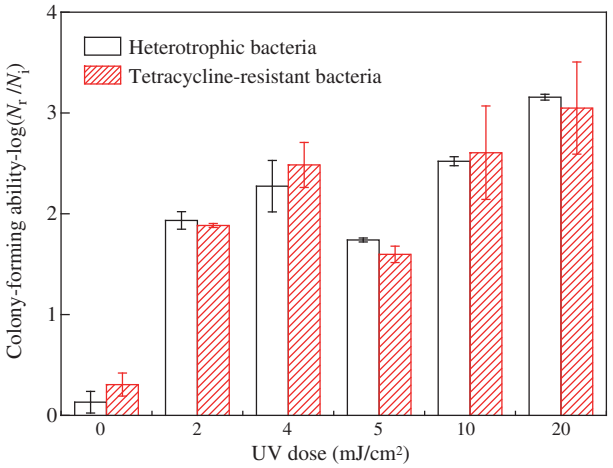

Fig. 2 – Reactivation of tetracycline-resistant bacteria and heterotrophic bacteria in the secondary effluent.

Table 1 – Maximum inactivation ratios, UVDs and inactivation rates of tetracycline-resistant isolates in the secondary effluent.

Strains	IR_{max} (40 mJ/cm^2)	UVDs (mJ/cm^2)	Inactivation rates K (/s) (±SE; r [b])
Aeromonas-1	5.26(±0.03) [a]	10	0.77(±0.05; 0.94)
Aeromonas-2	6.38(±0.39)	10	1.01(±0.02; 0.99)
Aeromonas-3	5.06(±0.10)	10	1.01(±0.07; 0.95)
Aeromonas-4	5.06(±0.13)	10	0.85(±0.03; 0.98)
Enterobacter-1	4.88(±0.21)	20	0.58(±0.07; 0.82)
Enterobacter-2	5.29(±0.43)	20	0.57(±0.04; 0.92)
Enterobacter-3	6.50(±0.21)	20	0.60(±0.04; 0.94)
Escherichia-1	6.08(±0.01)	10	0.68(±0.04; 0.97)
Escherichia-2	5.73(±0.09)	10	0.84(±0.05; 0.96)
Escherichia-3	6.44(±0.44)	10	0.70(±0.04; 0.95)
Escherichia-4	4.91(±0.31)	10	0.86(±0.04; 0.97)
Escherichia-5	6.10(±0.17)	10	1.09(±0.06; 0.96)
Citrobacter	4.98(±0.13)	10	1.04(±0.02; >0.99)
Hafnia	4.91(±0.15)	10	0.87(±0.07; 0.92)
Klebsiella-1	5.30(±0.06)	10	1.01(±0.06; 0.96)
Klebsiella-2	5.08(±0.09)	10	1.04(±0.05; 0.98)

[a] The inactivation ratio of tetracycline-resistant Aeromonas-1 was exposed at 20 mJ/cm^2.
[b] ±SD, intercept = 0.

resistant isolates was to indicate the tolerance to UV light. All data is shown in Table 1.

The maximum inactivation ratios of the isolates ranged from 4.9-log to 6.5-log. Aeromonas-3 and -4, Enterobacter-1, Hafnia, and Escherichia-4 were the most tolerant to UV light, while Aeromonas-2, Enterobacter-3, and Escherichia-3 were significantly less tolerant to UV light (Tukey's test, $p < 0.05$).

The UVD_Ss of Escherichia, Aeromonas, Klebsiella, Citrobacteria, and Hafnia strains were 10 mJ/cm^2. The UVD_Ss of the three Enterobacter were 20 mJ/cm^2. It was found that strains belonging to one genus had one UVD_S in this study. The results suggest that high UV dose was more effective to inactivate Enterobacter strains than the other thirteen strains, and that a UV dose higher than 20 mJ/cm^2 is necessary to control tetracycline-resistant Enterobacter in wastewater.

The inactivation rates of tetracycline-resistant isolates ranged from 0.57/s to 1.04/s. According to the Tukey's test, the 16 strains can be distributed into three groups (Fig. 3). Except for the intersection region of Escherichia-1 and -3, the inactivation rates of strains in group 1 were significantly lower than those in group 2 (Tukey's test, $p < 0.05$). Likewise, except for the intersection region with Hafnia, the inactivation rates of strains in group 2 were significantly lower than those in group 3 (Tukey's test, $p < 0.05$). These results suggest that the tetracycline-resistant Enterobacter strains were significantly more tolerant to UV light than other tetracycline-resistant strains isolated from the secondary effluent. Combining the results for the maximum inactivation ratio and UVDs, the tetracycline-resistant Enterobacter-1 strain was the most UV-tolerant strain among all the isolated strains.

The inactivation rates of tetracycline-resistant isolates were higher than those of aerobic spores of *Bacillus subtilis*, anaerobic spores of *Clostridium perfringens*, Adenoviruses, Rotavirus, protozoa, *etc.* as reviewed by Hijnen et al. (2006). However, the inactivation rates of the three tetracycline-resistant Enterobacter strains were similar to those of vegetative bacteria like *Salmonella typhi*, *Shigella sonnei*, *Legionella pneumophila* and *E. coli* O157, *etc.* (Hijnen et al. 2006). The tolerance of TRB to UV light should be taken into consideration when secondary effluent is exposed to conventional UV dosages (16–40 mJ/cm^2).

2.4. Reactivation (dark repair) of tetracycline-resistant isolates from the secondary effluent

The dark repair of tetracycline-resistant strains in the secondary effluent after UV disinfection is shown in Fig. 4. From the CFA results for tetracycline-resistant strains, only Aeromonas-1 and Enterobacter-3 had no significant reactivation after UV disinfection (One-way ANOVA, $p > 0.05$). Except for Aeromonas-1, Enterobacter-3, Escherichia-1 and -5, the reactivation of tetracycline-resistant strains significantly occurred when the UV dose reached 20 mJ/cm^2 or 40 mJ/cm^2 (One-way ANOVA, $p < 0.05$). The results indicate that significant reactivation of tetracycline-resistant strains takes place after a high conventional dose of UV exposure is applied in wastewater disinfection.

In effect, the reactivation of tetracycline-resistant strains leads to an overall decrease in the inactivation ratio, which induces an increase in survival of tetracycline-resistant strains in secondary effluent after UV disinfection with one day's storage or transportation. Hence, the inactivation ratios after 22 h dark repair were calculated to show the final inactivation of TRB and tetracycline-resistant strains in the secondary effluent when exposed to 20 and 40 mJ/cm^2 UV light, respectively (Table 2). The results show that only half of the 16 tetracycline-resistant strains reached 4-log inactivation after 22 h dark repair with 40 mJ/cm^2 UV disinfection in the secondary effluent. Remarkably, the final inactivation ratios of tetracycline-resistant Enterobacter-1 were

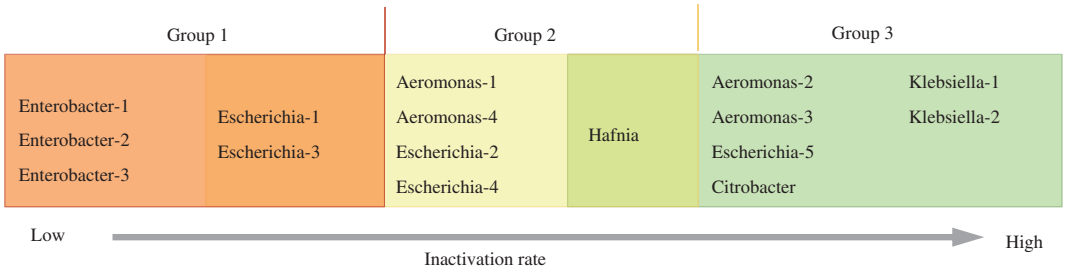

Fig. 3 – Groups of inactivation rates according to Tukey's test.

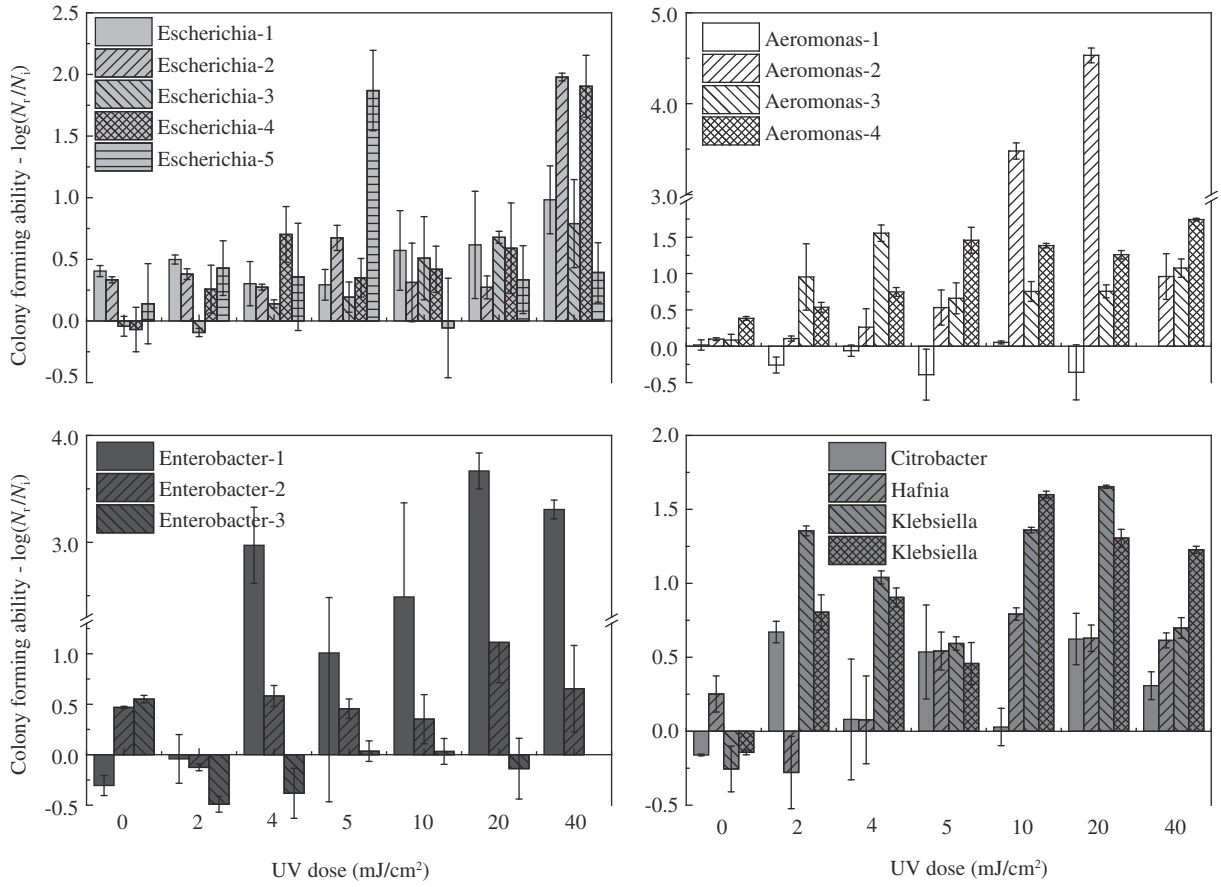

Fig. 4 – Reactivation of tetracycline-resistant strains in the secondary effluent. Asterisk means that the difference of colony forming abilities of the tetracycline-resistant strains between before and after UV disinfection is significant (One-way ANOVA, $p < 0.05$). UV: ultraviolet; ANOVA: analysis of variance.

as low as 1.18-log and 1.54-log with 20 and 40 mJ/cm^2 UV exposure after 22 hr incubation, respectively. The final inactivation ratio of tetracycline-resistant Enterobacter-1 was close to those of TRB and heterotrophic bacteria in the secondary effluent with 20 mJ/cm^2 UV exposure, which suggested that the final inactivation of bacteria depends on the lowest final inactivation of the species in the secondary effluent with UV disinfection. The results also suggest that strains with high reactivation potential would be a dominant contributor to the reactivation of the bacterial community in reclaimed water after UV disinfection. The reactivation of tetracycline-resistant strains increased levels of infectious Enterobacteria in the reclaimed water, and therefore increases microbial health risks, including gastroenteritis, during reclaimed water reuse.

3. Conclusions

The inactivation of TRB (3.0-log) was significantly lower than that of heterotrophic bacteria (>4.0-log) in the secondary effluent after exposure to 20 mJ/cm^2 UV light, which induced a significant increase in the proportion of TRB in the effluent. The inactivation rates of tetracycline-resistant strains isolated from the secondary effluent ranged from 0.57/s to 1.04/s. The tetracycline-resistant Enterobacter-1 was the most tolerant to

Table 2 – Real inactivation ratios of tetracycline-resistant strains after dark repair.

Strains	Real inactivation ratio (log) (±SD)	
	UV dose 20 mJ/cm^2	UV dose 40 mJ/cm^2
Aeromonas-1	5.62(±0.38)	–
Aeromonas-2	2.14(±0.08)	5.32(±0.31)
Aeromonas-3	3.83(±0.09)	3.97(±0.13)
Aeromonas-4	3.93(±0.06)	3.31(±0.02)
Enterobacter-1	1.18(±0.17)	1.54(±0.09)
Enterobacter-2	4.24(±0.40)	4.53(±0.43)
Enterobacter-3	5.35(±0.30)	5.65
Escherichia-1	5.07(±0.44)	5.09(±0.28)
Escherichia-2	4.86(±0.09)	3.75(±0.03)
Escherichia-3	3.67(±0.05)	5.53(±0.36)
Escherichia-4	4.76(±0.37)	2.93(±0.25)
Escherichia-5	6.04(±0.28)	5.69(±0.24)
Citrobacter	5.71(±0.17)	4.66(±0.09)
Hafnia	4.35(±0.09)	4.28(±0.05)
Klebsiella-1	3.71(±0.01)	4.60(±0.07)
Klebsiella-2	4.07(±0.06)	3.84(±0.02)
TRB in this study	1.18	–
Heterotrophic bacteria in this study	1.19(±0.03)	–

UV light among all strains. TRB, tetracycline-resistant isolated strains, as well as heterotrophic bacteria were found to be capable of reactivation (dark repair) in the secondary effluent, even when the UV dose reached 20 mJ/cm^2. The CFA of TRB and heterotrophic bacteria reached 3.2-log and 3.0-log under 20 mJ/cm^2 UV exposure after 22 hr incubation. The final inactivation ratio of tetracycline-resistant Enterobacter-1 was only 1.18-log under 20 mJ/cm^2 UV exposure after 22 hr incubation, which was close to those of TRB (1.18-log) and heterotrophic bacteria (1.19-log). The increased concentration of TRB and the reactivation of tetracycline-resistant enterobacteria in reclaimed water could pose a microbial health risk during wastewater reuse.

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