Original Article

Growth and Repair Potential of Three Species of Bacteria in Reclaimed Wastewater after UV Disinfection*

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Abstract

Objective The growth and repair potential of three typical microorganisms in reclaimed water after UV disinfection was investigated to assess the effects of photo-reactivation and dark repair of microorganisms, and the microbial safety of reclaimed water following this procedure.

Methods The growth and repair potential of *Escherichia coli*, a fecal coliform strain and *Bacillus subtilis* in the effluent of a biological wastewater treatment plant disinfected by a low-pressure UV lamp were investigated.

Results Any increase in bacterial numbers in the effluent after UV disinfection was due to damage repair. Exposure to photo-reactivating light for 8-10 h after UV irradiation with a dose of 5 mJ/cm^2, the highest percentage of photo-reactivation observed for *E. coli* and the fecal coliform strain was 29% and 15% respectively. *B. subtilis* showed little photo-reactivation under these conditions. The percentage of photo-reactivation was related to the UV dose and the photo-reactivating time, and a function was developed to forecast the final concentrations of *E. coli* and the fecal coliform strain after UV disinfection with possible photo-reactivation.

Conclusion Different species of bacteria displayed different responses to UV light and different repair potentials. The repair of indigenous bacteria in wastewater needs to be investigated in future work.

Key words: Wastewater reclamation; UV disinfection; Growth; Repair; Photo-reactivation; Dark repair

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INTRODUCTION

Wastewater reclamation and reuse is an effective approach for tackling the problem of water shortage[1]. Considering public safety, disinfection is a necessary process in wastewater treatment to reduce the risk of transmission of waterborne infectious diseases during water reuse[2]. Chlorination has been widely used, but questions have been raised regarding its toxic disinfection by-products (DBPs), the erosion of water pipes and unsafe operation. As an alternative technology, UV disinfection is gaining increasing popularity as it has been shown to effectively inactivate a wide range of pathogens, including the most problematic waterborne parasites, such as *Cryptosporidium* and *Giardia*, while forming fewer DBPs[3-4].

One disadvantage of UV disinfection is that it does not lead to continuous disinfection, and therefore does not prevent the increase of bacteria after UV disinfection. In fact, the increase in bacterial numbers in wastewater after UV disinfection treatment might be more significant for several reasons. Unlike drinking water disinfection, pathogens in the effluents of wastewater treatment

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plants may remain at a comparatively high level, according to its reuse purpose and the corresponding safety criterion. Since there are some nutrients left in the effluent water, growth of bacteria is possible. At the same time, exposure to light after UV disinfection is inevitable, so photo-reactivation deserves attention[5]. In addition, the long distances covered by reclaimed water may provide UV-irradiated microorganisms the opportunity to undergo repair processes. All possible growth and repair events threaten the safety of water reclamation.

In summary, there may be three factors responsible for the increase in microorganism counts after UV disinfection of wastewater. The first is the growth of uninjured microorganisms. The second is reactivation, and the third is the growth of these revived microorganisms. In previous studies, the concepts of 'photo-reactivation', 'dark repair', and 'growth' of bacteria have been confused. The role that each of these processes could play in the increase in bacterial numbers after UV disinfection is not clear yet. Moreover, since most previous studies used phosphate-buffered saline or distilled water, studies involving the effluent of wastewater treatment plants are necessary[5–20], along with investigations into the growth extent of uninjured and 'revived' microorganisms in wastewater after UV disinfection. Only after such investigations can the adequate control measures be proposed. The objective of this study was to investigate the potential growth and repair of three typical microorganisms in reclaimed wastewater after UV disinfection.

MATERIALS AND METHODS

Wastewater Samples

Water samples were collected from the secondary and tertiary effluents of a biological wastewater treatment plant in Beijing, China. The quality of the wastewater is described in Table 1. The wastewater was filtered through a 0.22 μm membrane filter to remove existing bacteria, providing a sterilized wastewater solution.

Bacterial Strains

Pure cultures of *Escherichia coli* strain CGMCC 1.3373, fecal coliform strain G215 and *Bacillus subtilis* strain CGMCC 1.73, provided by the Institute of Microbiology (Chinese Academy of Sciences, Beijing, China), were used as test microorganisms.

Table 1. The Quality of Secondary and Tertiary-treated Effluents

	COD (mg/L)	DOC (mg/L)	UV_{254}	Turbidity (NTU)	pH
Secondary Effluent	83–90	5.8–14.2	0.15–0.20	1.3–3.7	7.1–7.5
Tertiary Effluent	66–87	1.2–3.9	0.11–0.13	0.3–1.0	7.3–7.4

Note. COD: Chemical Oxygen Demand; DOC: Dissolved Organic Carbon; UV: Ultraviolet.

Cell Preparation

Fifty microliters of conserved culture of *E. coli* strain CGMCC 1.3373 were incubated in LB broth at 37 °C overnight until stationary phase was reached. The cells were collected by centrifugation (10 000 rpm, 10 min, 4 °C), washed twice with a sterilized saline solution (0.9%), and subsequently suspended in the prepared wastewater sample to achieve a concentration of approximately 10^5 CFU/mL.

Fecal coliform strain G215 was incubated at 44.5 °C. For *B. subtilis* strain CGMCC 1.73, the incubation time was prolonged until spores were formed, which was confirmed by dyeing and microscopy. Centrifugation was performed at 6 000 rpm and subsequent pasteurization was applied to inactivate the nonspore cells.

Microorganism Growth in Wastewater

Logarithmic-phase cells were inoculated into sterilized wastewater, placed at room temperature or their optimal growth temperature and monitored over time for culturability.

UV Source and Low-pressure UV Dose Determination

A specially-designed bench-scale collimated beam apparatus was used to irradiate samples. This apparatus contained a low-pressure (40 W) mercury UV lamp. The selected UV lamp was housed above a polyvinyl chlorine collimating tube (33 cm) that aided focusing of the UV beam onto the sample to be irradiated. A sketch map of the collimated beam apparatus is shown in Figure 1.

UV Disinfection Experiments

Fifteen milliliter water samples contained in a Petri dish were placed under the collimated tube, and stirred slowly during UV irradiation. The irradiance values were fixed throughout the experiment and UV doses were controlled by changing exposure times. All samples were exposed at room temperature (12-15 °C). After irradiation, 500 μL of the irradiated samples were removed,

serially diluted, and then plated in triplicate onto nutrient agar to determine organism numbers following exposure. The plates were incubated at 37 °C or 44 °C for 24 h, and analyzed by standard plate counting techniques.

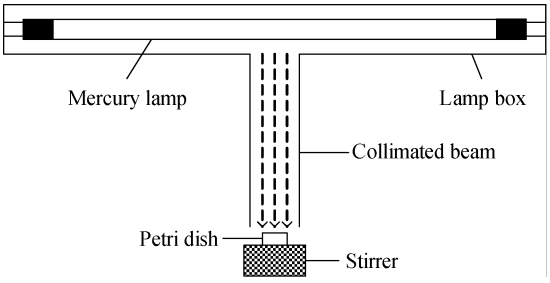

Figure 1. Sketch map of the collimated beam apparatus. The UV dose of the low-pressure mercury lamp was measured according to Bolton and Linden's method[21].

Photo-reactivation and Dark Repair Experiments

A fluorescent lamp (40 W) was used as the light source for photo-reactivation. The light intensity at 365 nm was 20 μW/cm^2[10]. Darkness for the dark repair process was ensured by covering the UV-irradiated water sample with silver paper. The experiment was conducted at room temperature. One milliliter sample was removed from each dish periodically for up to 72 h following the start of incubation and the samples were plated for enumeration.

Quantitative Evaluation of Photo-reactivation

To evaluate the effect of photo-reactivation, the percentage of photo-reactivation[6] was analyzed as follows: Percentage of photo-reactivation (%) = $(N_P–N)/(N_0–N) \times 100\%$

Where, N_P = cell number of the photo-reactivated sample (CFU/mL)

N = immediate survival after UV disinfection (CFU/mL)

N_0 = cell number before UV disinfection (CFU/mL)

RESULTS

Growth Potential in Secondary and Tertiary Effluent

The effluent of a biological wastewater treatment plant was used in this study to investigate potential bacterial growth. The growth of *E. coli*, the fecal coliform strain and *B. subtilis* in the secondary and tertiary effluents was investigated.

Figure 2 shows the growth of *E. coli* in the secondary and tertiary effluents under optimal conditions. The growth of *E. coli* in wastewater was not detected and bacterial numbers only varied slightly. In the secondary effluent, the number of *E. coli* increased from the initial inoculation of 4×10^3 CFU/mL to 9×10^3 CFU/mL during the 72-h incubation period, while bacterial numbers decreased to 10^3 CFU/mL after 72 h incubation in the tertiary effluent.

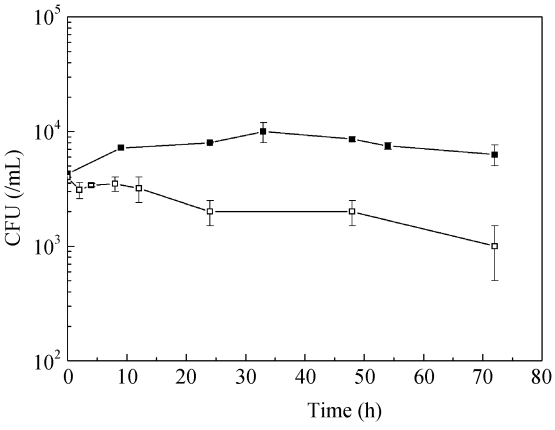

Figure 2. Growth of *E. coli* in the secondary (■) and tertiary (□) effluents of the wastewater treatment plant (T=37 °C, 130 rpm).

Similarly, growth of the other two species of bacteria (the fecal coliform strain and *B. subtilis*) tested in this study was also undetectable in wastewater during the 72-h incubation period at room temperature (data not shown).

Inactivation of Microorganisms by UV Treatment

Figure 3 shows the typical UV response curves of *E. coli*, fecal coliform strain and *B. subtilis*. It is clear that *E. coli* was the most sensitive to UV irradiation, fecal coliform was less sensitive and *B. subtilis* was the least sensitive to UV light because of the formation of spores. *E. coli* could be inactivated by more than 5-log after exposure to the UV dose of 25 mJ/cm^2, while 40 mJ/cm^2 was needed to reduce fecal coliform numbers by 5-log. Compared with these two species, *B. subtilis* was inactivated much more slowly, with a final inactivation rate of 4-log. When the UV dose was higher than 40 mJ/cm^2, no further change in the log reduction was evident, which was the same for all three species of bacteria.

Photo-reactivation in the Effluent

The potential photo-reactivation of the three species of bacteria in the tertiary effluent of the wastewater treatment plant was investigated (Figure 4).

After exposure to photo-reactivating light, samples exposed to 5, 20, 40, and 80 mJ/cm^2 of low-pressure UV irradiation showed different levels of photo-reactivation.

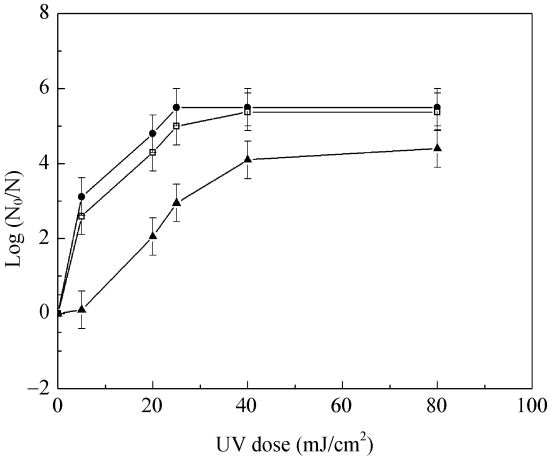

Figure 3. Log reduction (log(N_0/N)) of *E. coli* (●), fecal coliform strain(□), and *B. subtilis* (▲) with exposure to UV light.

E. coli was easily inactivated by UV light (as shown in Figure 3), and also showed obvious photo-reactivation (as shown in Figure 4(a)). The number of *E. coli* showed an immediate increase following exposure to photo-reactivating light. However, samples irradiated with different UV doses showed different levels of photo-reactivation. Following irradiation at a dose of 5 mJ/cm^2, the number of active *E. coli* cells per milliliter of water sample increased, and photo-reactivation reached its maximum level of nearly 10^5 CFU/mL at 8 h. This maximum level was much higher than the number of organisms that survived following immediate disinfection, and only a little lower than the initial concentration before disinfection (2×10^5 CFU/mL). While for the samples treated with UV doses higher than 5 mJ/cm^2, the cell number fluctuated around the detection limit during the 72 h incubation, indicating insignificant photo-reactivation.

The fecal coliform strain also showed some photo- reactivation potential, but to a less extent than *E. coli*. With a UV dose of 5 mJ/cm^2, fecal coliform photo-reactivation resulted in 2×10^4 CFU/mL at 2 h, higher than the survival rate following immediate disinfection (5×10^2 CFU/mL). Exposure of samples to light following UV irradiation at 20 mJ/cm^2 showed an increase from the initial photo-reactivation of 10 CFU/mL to a maximum of 10^2 CFU/mL at 24 h. The samples irradiated with higher UV doses (40 and 80 mJ/cm^2) showed undetectable photo-reactivation after exposure to light and the final bacterial numbers remained low.

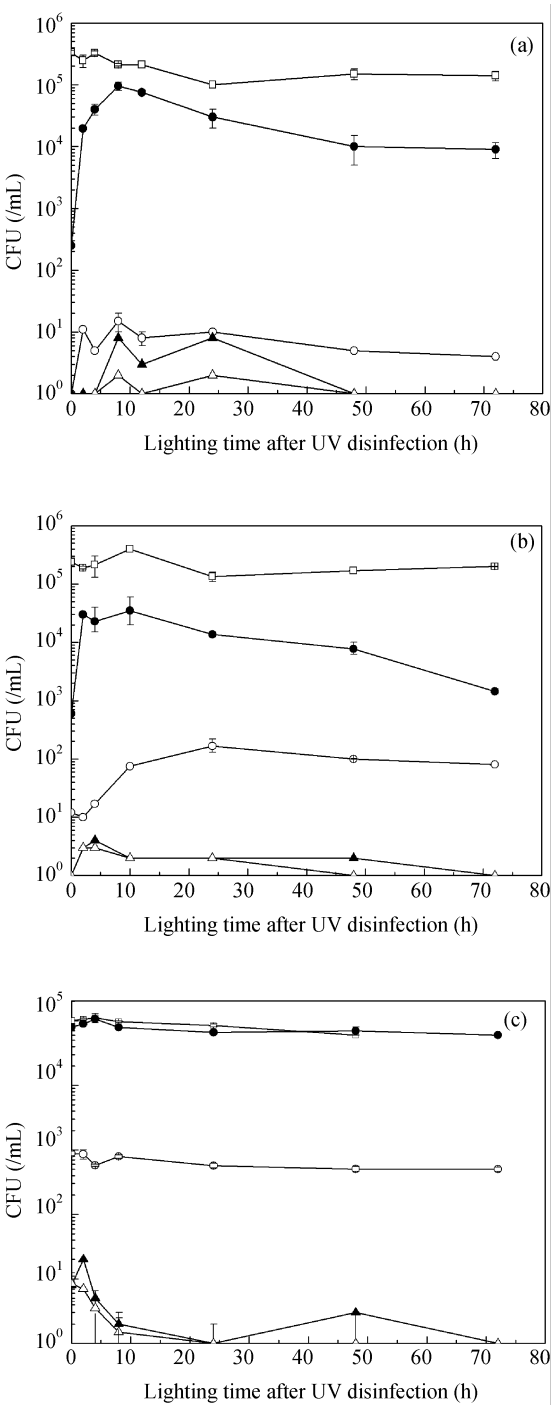

Figure 4. Photo-reactivation of *E. coli* (a), fecal coliform strain (b) and *B. subtilis* (c) in the tertiary effluent (□ no disinfection control; ● 5 mJ/cm^2; ○ 20 mJ/cm^2; ▲ 40 mJ/cm^2; Δ 80 mJ/cm^2).

B. subtilis presented little photo-reactivation potential. With different UV irradiation doses, survival of organisms following immediate disinfection was consistent during the 72-h incubation period. Bacterial numbers in samples treated with higher UV doses were maintained around the detection limit.

The photo-reactivation percentages of *E. coli* and fecal coliform strain were determined for different expositions and the data are presented in Tables 2 and 3. The highest photo-reactivation potential was observed at a dose of 5 mJ/cm^2. Following UV irradiation at a dose of 5 mJ/cm^2, a maximum photo-reactivation percentage of 28.73% was observed for *E. coli* after 8 h of light exposition, while a photo-reactivation percentage of 14.37% was observed for the fecal coliform strain at the same UV dose, under the same light incubation conditions. Exposure of samples to light following UV irradiation at 20, 40, and 80 mJ/cm^2 showed similar percentages of photo-reactivation both for *E. coli* and fecal coliform strain.

Table 2. The Photo-reactivation Percentage (%) of *E. coli* under Various Light Incubation Conditions

UV dose (mJ/cm^2)	Light exposure (h)				
	2	4	8	12	24
5	5.87	12.05	28.73	22.67	9.02
20	3.03×10^{-3}	1.21×10^{-3}	4.24×10^{-3}	2.12×10^{-3}	2.73×10^{-3}
40	0	0	2.12×10^{-3}	6.06×10^{-4}	2.12×10^{-3}
80	0	3.03×10^{-4}	6.06×10^{-4}	3.03×10^{-4}	6.06×10^{-4}

Table 3. The Photo-reactivation Percentage (%) of Fecal Coliform Strain under Various Light Incubation Conditions

UV dose (mJ/cm^2)	Light exposure (h)				
	2	4	8	12	24
5	12.28	12.28	14.37	5.46	12.28
20	0	2.08×10^{-3}	2.63×10^{-3}	6.46×10^{-3}	0
40	8.33×10^{-4}	1.25×10^{-3}	4.17×10^{-4}	4.17×10^{-4}	8.33×10^{-4}
80	8.33×10^{-4}	8.33×10^{-4}	4.17×10^{-4}	4.17×10^{-4}	8.33×10^{-4}

With the aim of forecasting repair ability, the *E. coli* photo-reactivation results were fitted into the following function:

The percentage of photo-reactivation (%) =

$$\frac{N_p - N}{N_0 - N}\times 100\% = 3.071D^{-4.349}\times t^{0.729}\times 100\%$$

n=43, R=0.994, R^2=0.988, R^2_{adj}=0.987, P<0.0001

Where, N_P=cell number of the photo-reactivated sample (CFU/mL);

N=immediate survival after UV disinfection (CFU/mL);

N_0=cell number before UV disinfection (CFU/mL);

D=UV dose (mJ/cm^2);

t=photo-reactivating light exposure time (h).

This function fit the experimental results of this study well (R^2=0.963), indicating that the percentage of photo-reactivation correlated to the UV dose and the time of photo-reactivating light exposure. Accordingly, the percentage of photo-reactivation of *E. coli* after disinfection over a certain period of time could be predicted. Since the organic carbon concentration was shown to have little influence on photo-reactivation, this function may be generally applicable for the effluent of wastewater treatment plants, although this needs to be confirmed by further testing.

Similarly, the photo-reactivation of fecal coliform strain detected in this study fitted the following function:

The percentage of photo-reactivation (%) =

$$\frac{N_p - N}{N_0 - N}\times 100\% = 0.706D^{-3.924}\times t^{0.984}\times 100\%$$

n=45, R=0.989, R^2=0.978, R^2_{adj}=0.976, P<0.0001

This indicated that the UV dose and the photo-reactivating light exposure time could be used to forecast bacterial performance after UV disinfection.

Dark Repair in the Effluent

Figure 5 shows the dark repair of these three species of bacteria in the tertiary treated effluent. Similar to the results of photo-reactivation, *E. coli* demonstrated the highest dark repair potential. Following a UV dose of 5 mJ/cm^2, maximal revival levels increased from 2×10^2 CFU/mL to 10^3 CFU/mL after 4 h incubation in the dark. Higher UV doses led to less dark repair, and the final concentration of *E. coli* was lower.

Fecal coliform strain and *B. subtilis* showed undetectable levels of dark repair in this study. The number of active cells per milliliter of water sample was virtually unchanged during the 72 h incubation period in the dark, with the small decrease probably resulting from natural cell death.

DISCUSSION

E. coli was used in this study as it is commonly

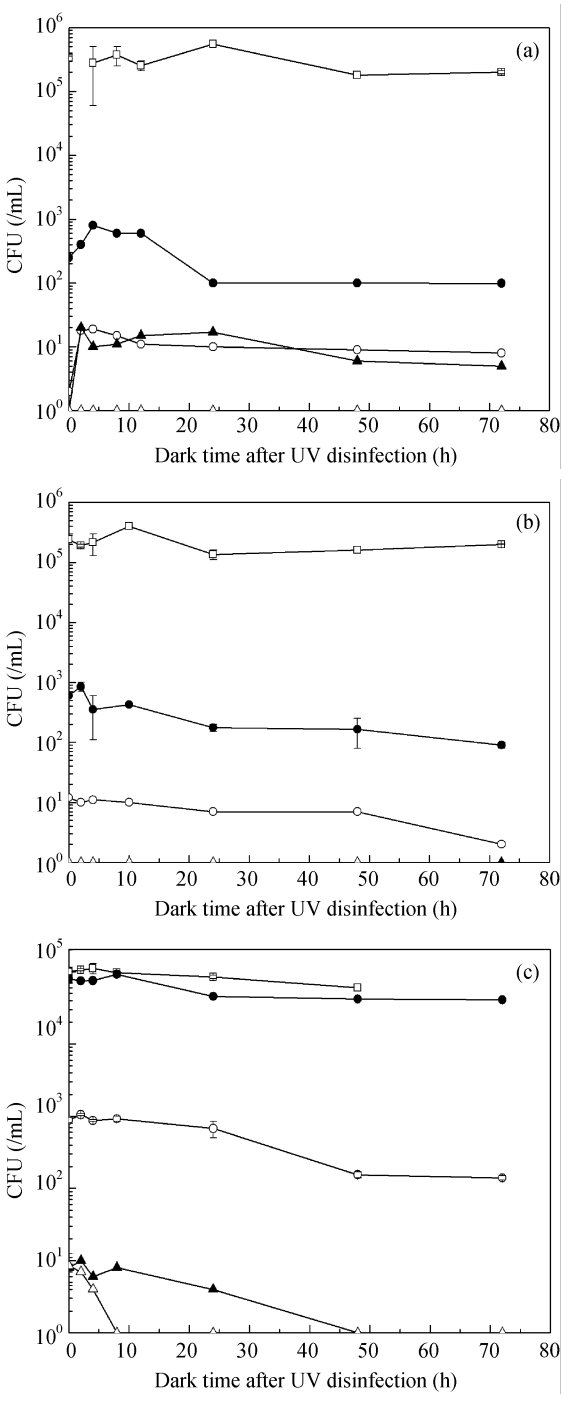

Figure 5. Dark repair of *E. coli* (a), fecal coliform strain (b), and *B. subtilis* (c) in the tertiary effluent (□ no disinfection control; ● 5 mJ/cm^2; ○ 20 mJ/cm^2; ▲ 40 mJ/cm^2; Δ 80 mJ/cm^2).

used as an indicator of disinfection efficiency in water systems and is known to undergo photo-reactivation following low-pressure UV exposure[22]. For wastewater disinfection, fecal coliforms number is another important indicator, while little was known about its repair mode after UV disinfection. *B. subtilis* is resistant to UV irradiation because of the formation of spores, and regarding its photo-reactivation ability, inconsistent results have been reported[6-7,17].

The microbial safety of wastewater after UV disinfection has aroused serious concern. Therefore, to introduce adequate control measures, the source of bacteria in UV-disinfected water needs to be investigated. The increased number of bacteria in UV-disinfected water results from three sources: the growth of uninjured bacteria, repaired bacteria and the growth of repaired bacteria. This indicates that growth and repair processes lead to an increase in bacterial numbers after UV disinfection, as expressed in the following formula:

$\Delta N_t = N_g + N_R + N_{Rg}$

where,

ΔN_t= the increased number of bacteria in wastewater after UV disinfection at time t following immediate disinfection (CFU/mL);

N_g= growth of uninjured bacteria (CFU/mL);

N_R= repair of injured bacteria (CFU/mL);

N_{Rg}= growth of repaired bacteria (CFU/mL).

The effect on bacterial growth and repair of UV disinfection of wastewater has not previously been investigated experimentally. It can be concluded from this study that the growth of bacteria in the secondary and tertiary treated effluents used in these experiments was not significant compared with the levels of bacterial repair discussed later. It was hypothesized that, as the secondary and tertiary effluents are the end products of biological wastewater treatment process, the assimilative nutrients contained in the effluent were limited, so the growth of bacteria, including uninjured and repaired bacteria in the effluent, was insignificant. In the proposed formula, N_g and N_{Rg} are negligible. Accordingly, the formula can be presented as:

$\Delta N_T = N_R$

This means that all of the increases in bacterial numbers detected in this study were due to the repair of injured bacteria. The repair of *E. coli*, fecal coliform strain and *B. subtilis*, including photo-reactivation and dark repair, differed in the effluents from the wastewater treatment plant, indicating different repair models between these organisms after different doses of UV disinfection.

For *E. coli* and fecal coliform strain, samples treated with a low UV dose (5 mJ/cm^2) displayed a

higher maximum level of photo-reactivation, compared with those treated with higher UV doses (20, 40, and 80 mJ/cm^2). *E. coli* showed a higher maximum level of photo-reactivation than the fecal coliform strain. Higher UV doses led to a lower "maximum" level of photo-reactivation, and the photo-reactivating exposure time required to reach this level was longer. This indicated that greater damage was induced by UV and thus longer time periods were required for repair. Since each microorganism harbors only approximately 20 photolyase enzymes and each enzyme can repair only approximately five dimers per min[21], it should be noted that bacterial numbers after repair can never reach the initial concentration of bacteria prior to UV irradiation, as previously reported[22–26]. This illustrates that some irreversible cell damage will occur and that complete repair is not possible. No detectable photo- reactivation of *B. subtilis* was observed in this study, supporting the findings of Lindenauer & Darby[6], but opposing those of Hassen et al.[7]. Limited photo-reactivation of *B. subtilis* may be due to its own sensitivity to exoteric pressure. The spores produced by *B. subtilis* help it to endure harsh conditions, including not only UV irradiation, but also light and dark exposure.

Compared with photo-reactivation, dark repair is a less significant form of repair. Only *E. coli* showed weak dark repair potential during the 72 h incubation period in this study. This may be due to the fact that the damage caused by UV light is more effectively repaired by another kind of light, for example, near ultraviolet or visible light. Energy is needed to repair damage. Although the levels of dark repair were not significant in this study, confirming previous findings[14,20,25], this process should not be disregarded, since given the time taken for the transportation of wastewater, dark repair still presents a potential risk.

As shown in Figure 3, *E. coli* and fecal coliform strain are more sensitive to UV light than *B. subtilis*. However, the results of repair analysis indicated that *E. coli* and fecal coliform strain display repair potential while the strain of *B. subtilis* tested did not (Figures 4 and 5). This indicated that bacterial repair weakens the effect of UV disinfection, leading to a potential safety risk. Consequently, the effectiveness of disinfection should be evaluated taking into consideration the total time from disinfection to the end user/environment, so as to ensure the safety of wastewater reuse.

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