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Efficacy of UVC-LED in water disinfection on *Bacillus* species with consideration of antibiotic resistance issue

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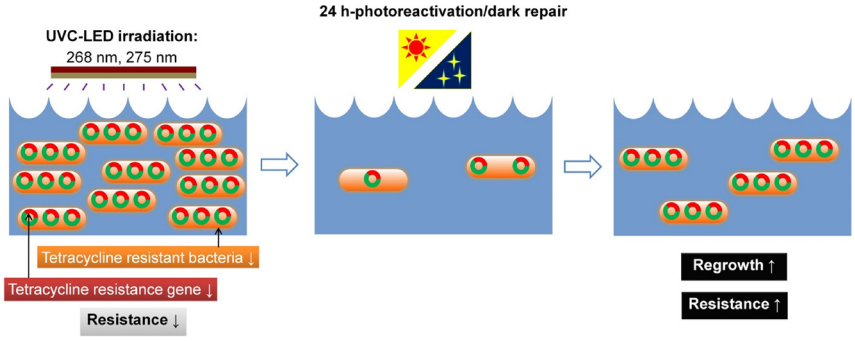
GRAPHICAL ABSTRACT



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ABSTRACT

Ultraviolet light emitting diode (UV-LED) has attracted extensive attention as a new technology to replace traditional mercury lamp for water disinfection. This study reported for the first time the application of UVC-LEDs in range of 200 – 280 nm for the treatment of two Gram-positive tetracycline resistant bacteria (TRB) from *Bacillus* species and their tetracycline resistant gene (TRG). The results showed that UVC-LEDs can inactivate TRB up to 5.7-log and inhibit TRG expression, especially at 268 nm. The required fluence was approximate to that of the referential non-resistant bacteria using the same UVC-LED, but far less than that of TRB using mercury lamp. After UVC-LED irradiation, photoreactivation was the dominant mechanism to repair TRB, just like non-resistant bacteria. But contrary to non-resistant bacteria, the regrowth ratio of TRB was remarkably high at 24 h since the end of the irradition, nevertheless the number of the regrown bacteria in the irradiated water was still less than that in the non-irradiated water. Whereas TRB restored resistance after repair even applying 268 nm at a fluence up to 46.08 mJ/cm^2 (maximum in this study). This study highlights the merits of UVC-LED to effectively inactivate TRB in a prompt, energy-efficient and resistance-reducing way, while future study on TRB regrowth and resistance resilience is needed.

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1. Introduction

A growing number of antibiotic resistant bacteria (ARB) and their associated antibiotic resistance genes (ARGs) has rapidly occurred in aquatic environment and even drinking water, due to incessant and extensive use of antibiotics in hospitals, household, livestock farm and aquaculture (Qiao et al., 2018; Xi et al., 2009). Along with vertical and horizontal gene transfer (HGT) in bacteria, the resistance can be evolved in pathogens and cause infections untreatable, which poses a serious threat to human health. Therefore, there is an urgent need to control the catastrophic outbreak of antibiotic resistance in modern water and wastewater industry. So far, most technologies have focused on the elimination of antibiotic compounds from wastewater, as antibiotic resistance will be induced when bacteria exposed to low-concentration of antibiotics for a long time (Cai and Hu, 2018; Shen et al., 2010). However, the study taking ARB and ARGs as treatment targets is relatively limited (Hiller et al., 2019; Sanganyado and Gwenzi, 2019; Sharma et al., 2016; Umar et al., 2019), which especially brings a critical challenge towards disinfection, the terminal step of water and wastewater treatment.

Since 1910, ultraviolet (UV) irradiation has been widely applied for water disinfection (Henry et al., 1910), as it will not produce toxic by-products that are usually generated from chemical disinfection (Liu et al., 2006). Traditional low pressure (LP) and medium pressure (MP) mercury lamps, emitting monochromatic light at 253.7 nm and polychromatic light at a broad range of 185 − 600 nm respectively, have been established and applied in full-scale disinfection practice around the world (Bolton and Cotton, 2008). Lately, A few LP mercury lamps were reported effective in reducing the concentrations of ampicillin-resistant (Pang et al., 2016) and tetracycline-resistant *Escherichia coli* (Childress et al., 2014) in wastewaters. In addition, the LP mercury lamp was found able to damage four ARGs both in extracellular form and present within four host ARBs belonging to *Staphylococcus aureus*, *Enterococcus faecium*, *E. coli*, and *Pseudomonas aeruginosa*, respectively (McKinney and Pruden, 2012). Besides, in another study about HGT between tetracycline resistant *E.coli* and non-resistant *E.coli*, it was found that LP mercury lamp did not affect the cell membrane permeability but directly damaged the plasmid containing ARGs, leading to the death of donor (or recipient), which demonstrates that the UV light might be advantageous over chemical disinfection strategies like chlorination in controlling ARGs transfer (Guo et al., 2015). However, the above study also showed that disrupting ARG required much greater fluence than inactivating ARB did. It means that the UV irradiation from mercury lamp is deficient in its capacity to destroy ARGs.

Recently, UV light-emitting diode (UV-LED) has risen to be a novel and credible UV light source to replace the traditional mercury lamps owing to its advantages such as free-mercury manufacture, flexible wavelengths, compact and durable device, short start-up time and low energy consumption (Ibrahim et al., 2014; Song et al., 2016; Li et al., 2019). As the DNA of most microorganisms is believed to have a maximum absorbance around 260 nm while the absorption spectrum of proteins shows a peak around 280 nm (Schmid, 2001), the UV-LED for inactivating microorganisms can be appropriate due to its diversity in wavelength, especially in the UVC range (200 − 280 nm) (Kowalski, 2009). Therefore, the UVC-LEDs have shown a potential in several water disinfection systems (Aoyagi et al., 2011; Lui et al., 2014, 2016; Nyangaresi et al., 2018; Vilhunen et al., 2009; Würtele et al., 2011; Oguma et al., 2013). Note that, in the aforementioned studies, all the testing microbial objectives are restricted to *E. coli*, virus and spores of *Bacillus subtilis* (Lui et al., 2014; Oguma et al., 2019), whilst they don't exhibit antibiotic resistance. Although the novel UV-LED technique is expected to be applicable in controlling antibiotic resistance, so far there is no record about their disinfection effect on ARB and ARGs in literature (Umar et al., 2019).

Meanwhile, a paradoxical phenomenon is that, the UV-damaged DNA can be recovered by way of two repair mechanisms: photoreactivation by the enzyme photolyase that requires light of 330 − 480 nm and nucleotide excision repair (dark repair) that is light independent (Friedberg et al., 1995; Harm, 1980; Kelner, 1949; Oguma et al., 2001). This was even observed for non-resistant *E.coli* in the UVC-LED systems in our previous studies (Nyangaresi et al., 2018, 2019b). Hence, it is also very important to study the repair characteristics if the damaged DNA of ARB or directly the ARG would be recovered after UVC-LED irradiation.

Therefore, this paper aims to look into the technical feasibility of the next-generation light source, UVC-LED, for photolytic disinfection on ARB in water. UVC-LEDs with emissions at 268 and 275 nm will be applied to inactivate two wild-type ARB that are screened from real wastewater. Both strains belong to Gram-positive (G^+) *Bacillus* species and concurrently tetracycline resistant bacteria (TRB). TRB and the associated tetracycline resistance gene (TRG) are of great interest because their occurrence is extremely high among all ARB and ARGs (Liu et al., 2019). The inactivation performance and the subsequent repair of two TRB are investigated, as well as the change of TRG and TRB's phenotypic resistance to tetracycline alongside this process. Based on the experimental results, the effectiveness of UVC-LED in controlling antibiotic resistance will be assessed comprehensively.

2. Materials and methods

2.1. TRB preparation

Two G^+ TRB strains isolated from the Qianpu Waste Water Treatment Plant, Xiamen were used as the treatment targets, with designation of *Bacillus cereus* TRB-3 and *Bacillus pumilus* TRB-5 (Boateng, 2014). Their details were depicted in Table S1. The TRB were inoculated in Luria-Bertani (LB) medium and incubated in a shaking incubator for 12 − 16 h at 30 °C. The bacterial solution was then line streaked on to tetracycline agar plates and incubated for 16 h at 30 °C to facilitate bacterial growth. From the bacteria grown on the plates, a single colony was isolated, and inoculated into 100 mL LB broth to mid-log phase via a shaking incubator for 4 − 6 h at 30 °C and 200 rpm. The cells were collected and washed with sterile saline solution (0.9 % NaCl) twice by centrifugation (10,000 rpm, 10 min) and re-suspended in sterile saline solution to obtain a final concentration of approximately 10^6 CFU/mL.

2.2. Identification and quantification of TRGs

Six types of TRGs (*tet*(A), *tet*(B), *tet*(K), *tet*(L), *tet*(Q) and *tet*(X)) was qualitatively detected by the polymerase chain reaction (PCR) to analyze the TRGs carried within the selected TRB strains. Plasmid DNA was extracted from each strain using the Plasmid Mini Kit I(Omega Bio-Tek, Inc., USA) following the manufacturer's protocol, and used to carry out the normal PCR as described in Supplementary Material.

Depending on their presence, the expression level of *tet*(A), *tet*(B), *tet*(K), *tet*(L), *tet*(Q) and *tet*(X) under UVC-LED irradiation will be analyzed via quantitative real-time polymerase chain reaction (qRT-PCR) method. The 16S ribosomal RNA (rRNA) gene would also be analyzed in order to normalize the abundance of TRGs in the collected samples (Rao et al., 2013). To accomplish this task, the total RNA was first extracted from the bacteria after irradiation using Takara MiniBest Universal RNA Extraction Kit (Takara Bio. Inc., China), and then cDNA was obtained through reverse transcription of the RNA via Transcript All-in-One First Strand cDNA Synthesis Supermix (TransGen Biotech Co. Ltd., China), following manufacturer's protocols. Finally, to quantitatively detect the changes of gene expression in the bacterial samples (both the control and the UVC-LED exposed ones), qRT-PCR was performed and the fold change for the target gene expression was calculated as detailed in Supplementary Material. The information of all PCR primers and annealing temperatures for the TRGs is shown in Table S2.

2.3. Assessment of antibiotic resistance phenotype

The tetracycline resistance of the tested strains was determined by minimum inhibitory concentrations (MICs) attained through broth microdilution (BM) with the dilution series extending from 1 to 256 mg/L (serial two-fold dilution) in accordance to the standards set out by the Clinical and Laboratory Standards Institute (Cockerill et al., 2013). The antimicrobial susceptibility tests by BM were carried out in a sterile 96-well cell microtiter plates (NEST Biotechnology Co. Ltd., China). The mixture, consisting of the TRB solution (approx. 5×10^5 CFU/mL), nutrient broth and tetracycline of different concentrations, was incubated at 37 °C for 16 – 20 h. Note that, one well without tetracycline in each section was used as a growth control. Growth was indicated by a change in turbidity and the MIC was selected as the lowest concentration of antibiotic that prevented the visible growth of bacteria. Specifically for tetracycline, the MIC threshhold and interpretive criteria were defined as ≤ 4 mg/L (susceptible) and ≥ 16 mg/L (resistant) (Cockerill et al., 2013).

2.4. Measuring the fluences of UVC-LEDs

UVC-LEDs with emissions at 265 and 275 nm and optical power of 1.1, 1.6 mW respectively at currents of 20, 20 mA were achieved at voltages of 5.8, 5.4 V respectively (Great Bright Company, China). The emission spectra of the UVC-LEDs were measured using a Spectro 320 Optical Scanning Spectrometer (AMETEK Commercial Enterprise Co. Ltd., China) and exhibited peak emission wavelengths of 268 and 275 nm with full widths at half-maximum (FWHM) of approximately 12.5 and 10.5 nm respectively (Fig. 1a). The batch reactor contained nine UVC-LEDs with three rows and three columns in 24 mm square array, a magnetic stirrer, a 2400 Keithley Source meter and a microbial sample in a 60 cm diameter petri dish (Fig. 1b). The emission power of single 268 and 275 nm UVC-LEDs with variation of driving current up to the optimum (20 mA as stated by the manufacturer) was measured by an integrating sphere with the UVC-LEDs fixed in an LED-850 TEC temperature control to maintain the solder temperature. The variation of power with current is shown in Fig. 1c. Fluence rate was measured at the same microbial suspension surface level using IL-1700 radiometer with SED 270 detector (International Light, USA). An equivalent fluence rate of around 0.38 mW/cm^2 was used that was achieved by driving the 268 and 275 nm UVC-LEDs at constant currents of 20 and 17 mA, respectively using a 2400 Keithley Source meter. The petri factor, water factor, divergence factor and reflection factor of the UV-LEDs are shown in Table S3. The fluence was a product of fluence rate and exposure time, t (s) (Bolton and Linden, 2003).

2.5. UVC-LED irradiation and inactivation experiments

All the powered UVC-LEDs were maintained at around 26 °C. The UVC-LEDs were powered on for 10 min before irradiation to reach a stable emission stage. 20 mL of microbial suspension was obtained from the re-suspended bacterial cells, 5 mL was taken away for initial bacteria count and the rest 15 mL was placed in a petri dish with 60 mm diameter (6 mm water depth) for irradiation. While being stirred with a sterile magnetic stir bar, the microbial suspension was irradiated at 20 mm from the UVC-LED source. Then 5 mL irradiated sample was taken to obtain the number of CFU/mL. Meanwhile the rest (10 mL) was used for the repair experiments. For changing different samples that were irradiated at different fluences, the UVC-LEDs were powered off for less than 30 s which did not have a significant effect on the fluence rate.

The inactivation efficiency of bacteria was analyzed by calculating log inactivation using Eq. (1).

$$\text{Log inactivation} = \text{Log}\left(\frac{N_0}{N}\right) \quad (1)$$

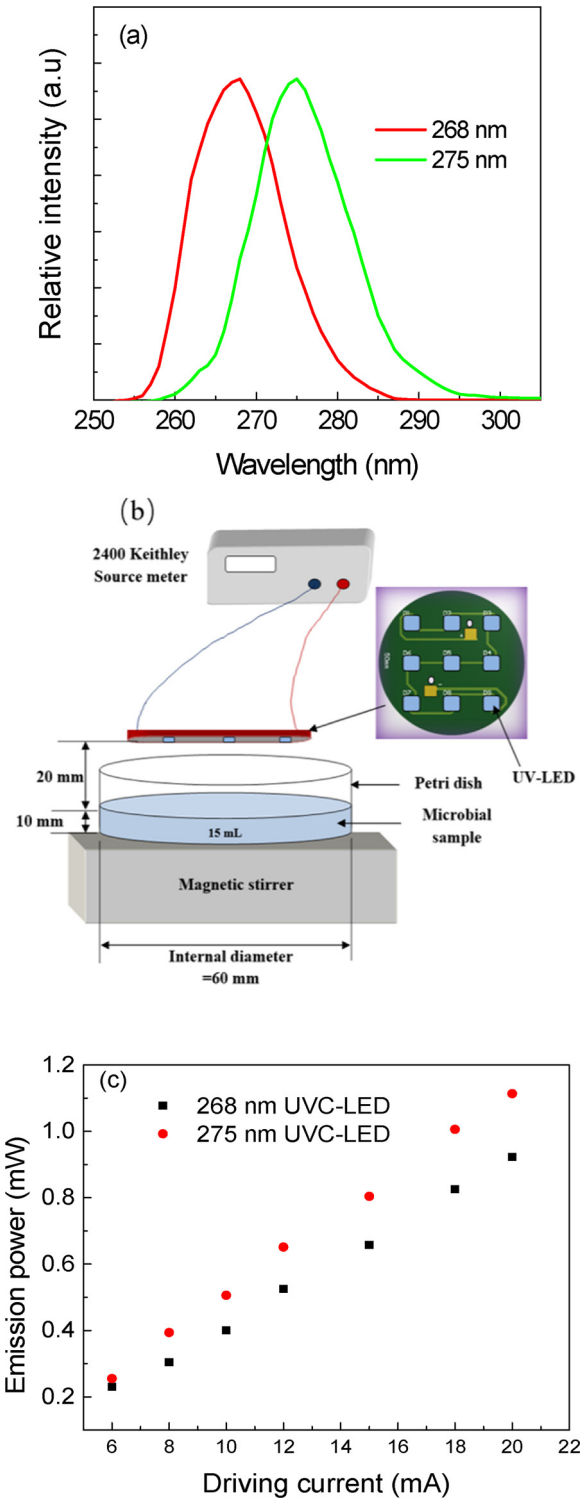

Fig. 1. Experimental conditions of UVC-LEDs: (a) emission spectra from the 268 nm, 275 nm; (b) set-up of a batch reactor and (c) emission power versus driving current.

in which, N_0 and N are the colony count (CFU/mL) before and immediately after disinfection, respectively.

2.6. Repair experiments

Samples for photoreactivation were taken immediately after UVC-LED irradiation and placed near to a transparent window for 24 h,

under exposure to natural light in similar clear weather conditions and constant room temperature. Although it is known that variation of light intensity affects the photoreactivation rate within one day, our case aimed at investigating what experience the TRB in the UV disinfected water would undergo with natural light in practice. Meanwhile, samples for dark repair were taken in tubes covered with aluminum foil after UVC-LED irradiation and kept in a cardboard box over the same time period (24 h). The serial dilution and plating were done at 2, 4, 8, 12 and 24 h, to evaluate the extent of repair. All repair samples were subjected to room temperature (25 ± 1 °C).

The percentage of photoreactivation and dark repair of TRB was quantified using Eq. (2) (Lindenauer and Darby, 1994).

$$\text{Percentage of repair (\%)} = \frac{N_t - N}{N_0 - N} \times 100\,\% \quad (2)$$

in which, N_t is the concentration of microorganisms after photoreactivation/dark repair for a period of time, t (h) (CFU/mL). N_0 and N have the same meaning as in Eq. (1).

To indicate the regrowth potential of bacteria after irradiation and repair, the growth ratio was expressed as Eq. (3) (Kashimada et al., 1996). For the reference TRB without irradiation, N_t specially represents the cell concentration after the same period of cultivation as the repair abided.

$$\text{Growth ratio (\%)} = \frac{N_t}{N_0} \bullet 100\,\% \quad (3)$$

2.7. Statistical analysis

All experiments were conducted in triplicates. Origin 8.6 was used to calculate the average and standard deviation so as to compare the results of TRB inactivation and repair (photoreactivation and dark repair) under different UVC-LED conditions, as well as the TRG fold change expressions after inactivating TRB with varying UVC-LED fluences.

3. Results and discussion

3.1. Effect of UVC-LED on TRB inactivation

The inactivation efficiencies of UVC-LED on TRB-3 and TRB-5 were displayed in Fig. 2. The results showed that, 268 nm afforded higher level of inactivation than 275 nm UVC-LED for both strains, being similar to previous reports on non-resistant bacteria inactivation by UVC-LEDs (Li et al., 2017; Nyangaresi et al., 2018). These results can be understood since the maximum light absorption for DNA of most bacteria is within 260 − 270 nm range (Schmid, 2001).

As observed in Fig. 2, the fluences to cause complete inactivation (no bacteria detected) of TRB-3 were lower than that of TRB-5, regardless of using 268 or 275 nm UVC-LEDs. In other words, TRB-5, a strain of *B. pumilus*, was found to be more resistant to UVC-LED disinfection than TRB-3. This is because *B. pumilus* possess much higher resistance to UV radiation than other species of *Bacillus* and thereby enables survival under standard disinfection practices (Gioia et al., 2007; Kempf et al., 2005; Newcombe et al., 2005; Setlow, 2006).

As shown in Fig. 2, the variation of inactivation with respect to fluence is similar with those obtained in earlier studies about ARB inactivation by mercury lamp (Zhang et al., 2017) and non-resistant bacteria inactivation by UVC-LED (Nyangaresi et al., 2018), demonstrating similar mechanisms in both lamp and UVC irradiation. Quantitatively, a fluence of 15.36 mJ/cm^2 by both 268 and 275 nm UVC-LEDs is able to inactivate TRB-3 completely (5.4-log reduction), while TRB-5 could be completely inactivated (5.7-log reduction) by 23.04 and 30.72 mJ/cm^2 at 268 and 275 nm, respectively. In our previous study using the same UVC-LED for inactivating non-resistant *E. coli*, 11.52 and 23.04 mJ/cm^2 by the 267 and 275 nm UVC-LEDs were required to obtain a complete 4-log inactivation (Nyangaresi et al., 2018), indicating a similar energy consumption to that of TRB complete inactivation. On the contrary, however, Huang et al. (2016) reported a higher flunce (40 mJ/cm^2) to yield a 4-log inactivation of the TRB compared with a lower fluence (20 mJ/cm^2) to instigate the same 4-log inactivation of non-resistant heterotrophic bacteria. In their study, the disinfection target for both TRB and non-resistant bacteria samples was a mixture of numerous bacteria from secondary effluent, and LP mercury lamp emitting UV light at 254 nm was used. For isolated bacteria strains, Huang et al. (2016) also proved that the UV tolerance of different bacterium varied greatly, as the different fluence-response of TRB-3 and TRB-5 shown in this study. It again reminds us that the direct effects or fluence needed to bring about inactivation should be dependent strongly on specific microbe (Linden et al., 2001; Vilhunen et al., 2009). This should be important for UVC-LED implementation in water disinfection when considering the unique feature of ARB.

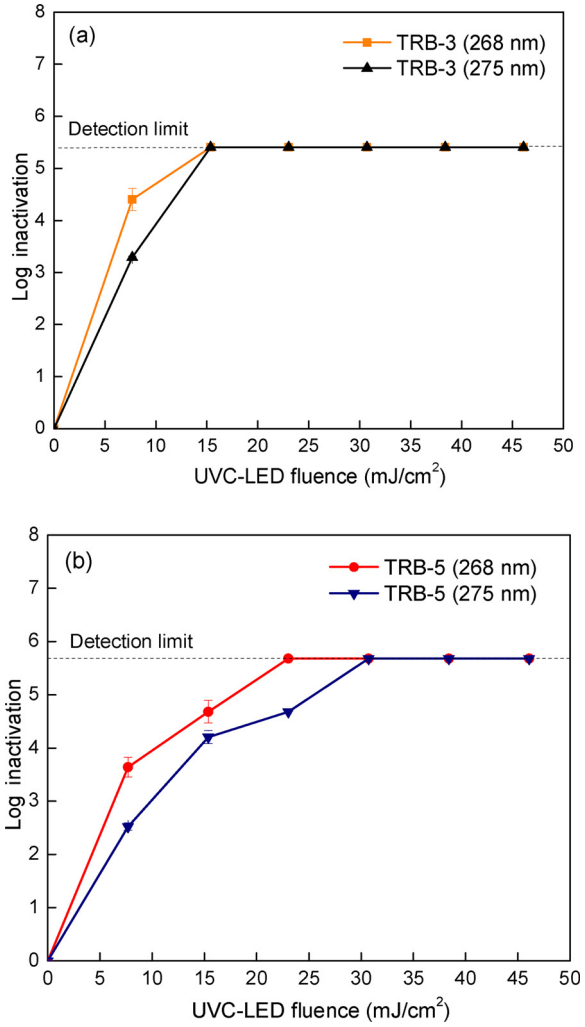

Fig. 2. Log inactivation of TRB-3 (a) and TRB-5 (b) after UVC-LED treatment at 268 nm/275 nm and different fluences.

3.2. TRB repair after UVC-LED irradiation

Generally, the higher fluence of UVC-LED applied to treat TRB, the less photoreactivation was observed (Fig. 3). When comparing the dependence of photoreactivation on the inactivation wavelength (Fig. 3a *vs.* b, c *vs.* d), it was found that the percentage of photoreactivation after inactivation by 268 nm was slightly less than that of 275 nm. Contrarily, 275 nm UVC-LED was reported to have a lower repair for non-

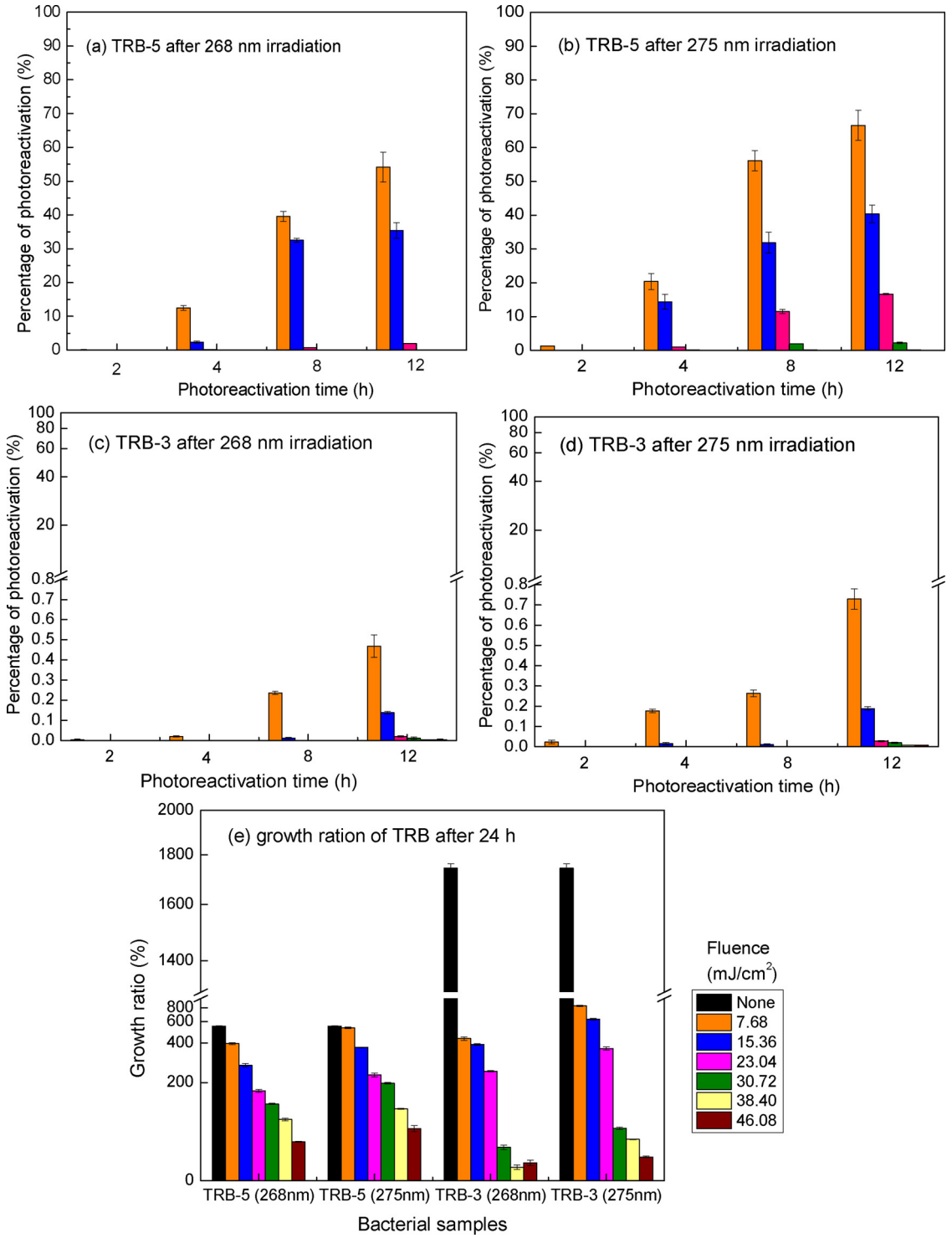

Fig. 3. Photoreactivation of TRB with different UVC-LED conditions. (a) TRB-5 after 268 nm irradiation; (b) TRB-5 after 275 nm irradiation; (c) TRB-3 after 268 nm irradiation; (d) TRB-3 after 275 nm irradiation; (e) growth ratio of TRB at 24 h after irradiation.

resistant bacteria in latest studies (Nyangaresi et al., 2018, 2019a). This result once again indicates the unique physiological properties of ARB in view of the response to UV irradiation. Basically, UV disinfection can inactivate microorganisms mainly because mutagenic DNA lesions like cyclobutane pyrimidine dimmers (CPDs) are induced from UV irradiation with a maximum absorption around 260 nm. When CPDs are monomerized by photolyase with light of 330 – 480 nm, the inactivated bacteria can be recovered (Eker et al., 1990; Oguma et al., 2001). In nature, photolyase is a large family of enzymes and can be widely found in bacteria as an adaption to UV light exposed habitat (Goosen and Moolenaar, 2008). Thus the damage caused by 268 nm UV on DNA should be more readily repaired than that by 275 nm UV on protein for most bacteria. Meanwhile for ARB, the resistance-associated proteins within bacterial cells are usually very large (> 15 000 Da) and need to be expressed by various antimicrobial resistance coding genes (Zhu et al., 2018), which implies that the damaged DNA sites of ARB may be much more complicate and diverse than that of the non-resistant bacteria under the same 268 nm irradiation. In turn, the recovery of all

damaged DNA would be more difficult for ARB than the non-resistant bacteria after 268 nm UV irradiation.

Further, as shown in Fig. 3a *vs.* c, b *vs.* d, TRB-5 is more prone to be photoreactivated than TRB-3. This confirms that TRB-5 is more resistant to UV irradiation as discussed in the former section and agrees with previous reports that *B. pumilus* is highly robust to environmental pressure in literature (Gioia et al., 2007; Kempf et al., 2005; Newcombe et al., 2005; Setlow, 2006). While, no matter there was a gradual increase (TRB-5, Fig. 3a and b) or marginal variance (TRB-3, Fig. 3c and d) in the photoreactivation percentage throughout the first 12 h, a substantial increase appeared from 12 to 24 h as the growth ratio indicated (Fig. 3e), with most of the samples even surpassing 100 %. Further, the control samples that were not subjected to UVC-LED irradiation experienced an approximate 3.6-fold and 16.4-fold increase after a 24 h period in natural light with respect to TRB-5 and TRB-3, respectively. That high photoreactivation percentage did not occur in our previous study on non-resistant *E.coli* using the same irradiation conditions (Nyangaresi et al., 2019b). However, the results agree with a study carried out by Childress et al. (2014). Based on their overall findings, the common UV irradiation in wastewater treatment plant is effective in reducing the concentration of TRB. But in their case, regrowth happens and eventually increases to a point where it is greater even than the initial concentration of bacteria. It therefore points to the importance of further study on UVC-LEDs optimization to minimize the regrowth of ARB after irradiation. Nevertheless, by comparing the control sample without disinfection, the increasing fluence and 268 nm in UVC-LED are found to be advantageous to suppress the photoreactivation of ARB for a long duration of 24 h (Fig. 3e). Specifically, the minimum growth ratio at 24 h for TRB-3 and TRB-5 could be decreased to 17 % (by 268 nm, 38.04 mJ/cm^2) and 55 % (by 268 nm, 46.08 mJ/cm^2), respectively.

A similar recovery pattern occurred for 24 h of dark repair (Fig. 4), but the degree was less than the photoreactivation. It thereby suggests that photoreactivation is the dominant DNA repair mechanism for TRB after UVC-LED treatment. This finding was in agreement with other non-resistant bacteria recorded in literature, after UV irradiation with either mercury lamp or UVC-LED (Auffray and Boutibonnes, 1987; Nyangaresi et al., 2018; Oguma et al., 2001). After UVC-LED irradiation, the counts of TRB-3 and TRB-5 have reached the detection limit (Fig. 2). But this does not mean that all bacteria have been inactivated. Therefore, the percentage of photoreactivation and dark repair may be caused by the regrowth of the remaining culturable TRB-3 and TRB-5 after UVC-LED disinfection.

3.3. Effect of UVC-LED on TRG expression level

Different types of TRG were assessed using PCR, to give rise to a resistance “fingerprint” for each bacterial sample (Table S4). The tetracycline resistance of the bacteria in this study was found to be inferred by the presence of *tet* determinant, *tet*(L). Notably, it was expected that the results would have shown positive for *tet*(L), as tetracycline resistant *B. cereus* has previously been known to carry this gene on a plasmid (Agersø et al., 2002; Bernhard et al., 1978). Moreover, *tet*(L) have been found on plasmids, and/or in the chromosome of other species of *Bacillus* (Eccles and Chopra, 1984; Ives and Bott, 1989; Levy et al., 1989; Phelan et al., 2010; Roberts, 1996). Thereafter, the quantification of the gene copy numbers of *tet*(L) was carried out via qPCR and then the copies of *tet*(L) gene was normalized to the copies of 16S rRNA genes for each of the treatments following UVC-LED irradiation. Since it is impossible to calculate the relative abundance of ARGs in pure culture bacteria, the expression level of *tet*(L) was quantified in this way, as displayed in Fig. 5. The results showed that the expression level of *tet*(L) decreased after UVC-LED disinfection, from the initial amount (horizontal dashed line on each figure) present before irradiation. It suggests that UVC-LED disinfection has the potential to effectively inhibit ARGs’ expression (Venieri et al., 2017). In Fig. 5, the highest reduction of *tet*(L) expression, i.e. a 0.93 and 0.95 fold-decrease, was observed in the 268 nm UVC-LED disinfection with fluences of 38.4 and 46.08 mJ/cm^2, for TRB-3 and TRB-5, respectively. Although these fluencies can cause nearly 100 % inactivation of TRB-3 and TRB-5 (Fig. 2), the resistance dominant *tet*(L) remains expression. In previous studies using 254 nm UV mercury lamp, Zhang et al. (2017) reported similar findings whereby just a slight reduction (1.18-log) of their tetracycline determinant, *tet*(B), occurred in antibiotic-resistant *E. coli* strains being subjected to a high fluence of 80 mJ/cm^2. The fact that the ARG damage needs a far greater fluence than what required for bacterial inactivation indicates a more complex cell response to UV light rendering ARG destory than ARB inactivation (McKinney and Pruden, 2012).The expression of residue ARGs after UV irradiation should also be concerned as antibiotic resistance may spread by way of HGT. While, UVC-LEDs still exhibit superiority over tradition mercury lamp to suppress ARG expression with higher efficiency and lower energy consumption.

3.4. Effect of UVC-LED on TRB resistance phenotype

Fig. 6a showed the MIC values for two TRB strains after subjection to varying fluences of UVC-LEDs. At initial point of fluence zero, it shows that the original TRB-5 and TRB-3 are highly resistant against tetracycline, demonstrated by MIC value (256 mg/L) far beyond the threshold of tetracycline indicating resistance (16 mg/L) (Cockerill et al., 2013). After exposure to a fluence of 7.68 mJ/cm^2, only surviving TRB-5 from 275 nm UVC-LED maintained its same high level of resistance. In other cases, TRB have changed to “intermediate resistance” as MIC values fall into 8 − 16 mg/L. With increasing fluence, resistance of the irradiated TRB decreased as stepwise falling MICs shown in Fig. 6a. Once the fluence reached 23.04 mJ/cm^2, no growth of all TRB samples was observed in the standard MIC assay, which accounts for the absence of MIC values under those treatment conditions. These results show that UVC-LEDs disinfection can cause a dramatic change in the MICs of both TRB-3 and TRB-5, implying a sudden shift from being very resistant to the antibiotic to very susceptible by the application of a fluence of 23.04 mJ/cm^2. When comparing to LP mercury lamp, Zhang et al. (2017) documented the variations (both increase and decrease in the resistance to various antibiotics) with regards to antibiotic-resistant *E. coli* strains after 254 nm UV irradiation. However, their finding was mainly in accordance to other antibiotics tested (ampicillin, streptomycin, gentamycin, cefotaxime, chloramphenicol, ciprofloxacin and norfloxacin), whereas most of the treatments with tetracycline and sulphamethoxazole maintained their resistance after UV irradiation at a fluence of 80 mJ/cm^2. Cockerill et al. (2013) also recorded that there was no change in the antibiotic resistant profiles of the surviving ARB following UV disinfection. It therefore demonstrates that UVC-LEDs outperform traditional mercury lamp to reduce antibiotic resistance of ARB through disinfection.

Surprisingly, Fig. 6b showed that the MIC was restored for all the recovered TRB after 24 h photoreactivation and dark repair, despite the irradiation conditions in which TRB-5 and TRB-3 were subjected. Though after inactivation the number of TRB decreased considerably, after repair, regrowth and probable HGT from residue *tet*(L), both TRB strains regained high level of tetracycline resistance, which raises a serious concern regarding the control of antibiotic resistance when applying UVC-LED disinfection in the long run.

4. Conclusions

The results indicate that UVC-LEDs (268 and 275 nm) are effective in inactivating TRB, with the germicidal effectiveness of 268 nm being higher than 275 nm. Moreover, *B. cereus* TRB-3 displayed a higher inactivation potential compared to that of *B. pumilus* TRB-5, reflecting a microbe-dependent fluence-response in UV inactivation. However, despite nearly total inactivation, these two TRB can repair within 24 h

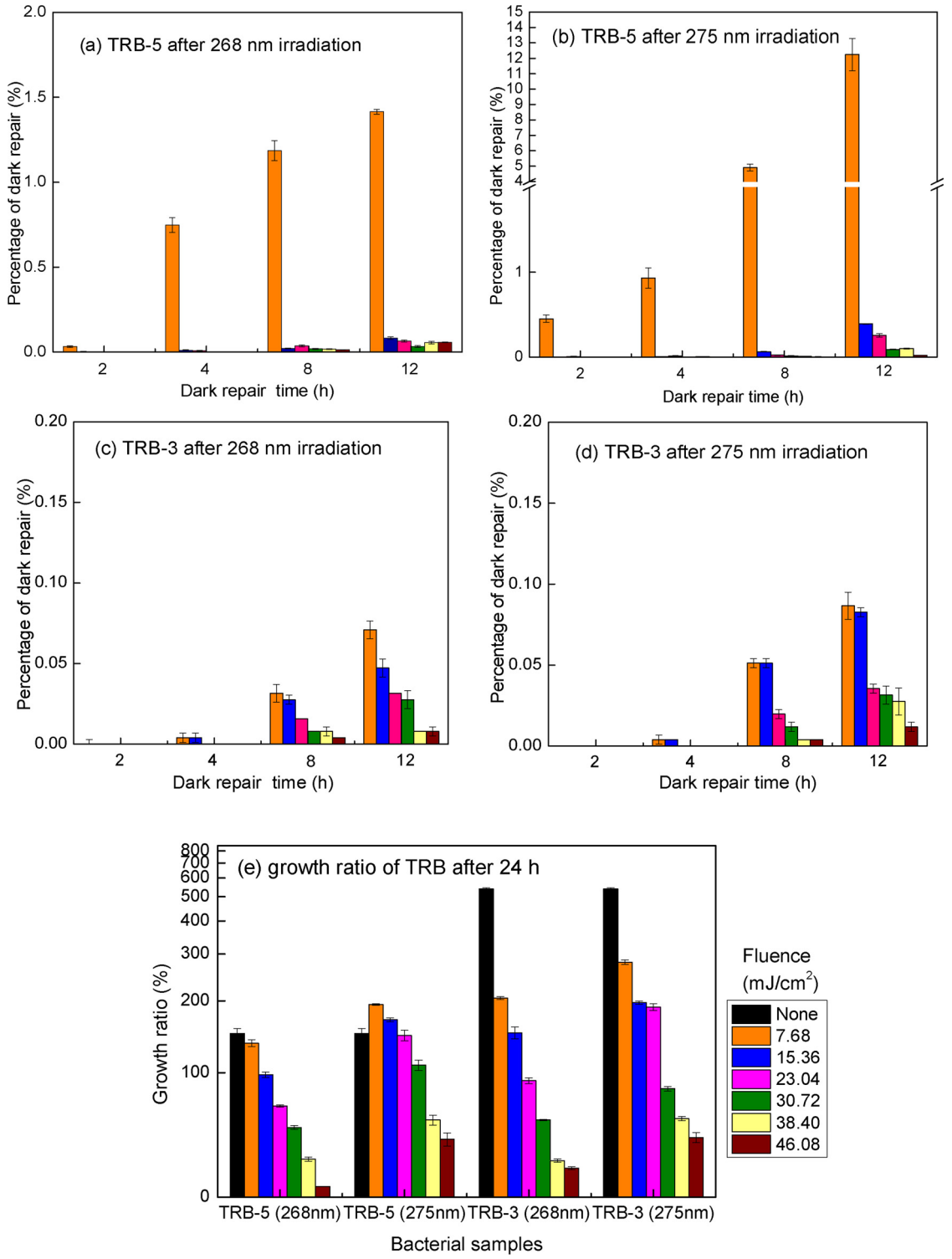

Fig. 4. Dark repair of TRB with different UVC-LED conditions. (a) TRB-5 after 268 nm irradiation; (b) TRB-5 after 275 nm irradiation; (c) TRB-3 after 268 nm irradiation; (d) TRB-3 after 275 nm irradiation; (e) growth ratio of TRB at 24 h after irradiation.

after UVC-LED irradiation, mainly resulted from photoreactivation. The regrowth even surpassed 100 % at low fluences. The resistance of TRB apparently showed a dramatic decrease (becoming susceptible to tetracycline) after irradiation by UVC-LEDs but was restored after repair. This is probably because UVC-LEDs failed to render a complete inhibition of TRGs like *tet*(L) and in turn spread antibiotic resistance by way of HGT (Platteeuw et al., 1995), or the prompter gene may be repaired during photoreactivation if TRG locates on the chromosome. Therefore, UVC-LED disinfection is an efficient and instant measure to inactivate ARB, reduce ARG and minimize resistance compared to mercury lamp, but not a once-and-for-all solution to eliminate risks of antibiotics resistance dissemination, especially due to the ARGs resilience and ARB regrowth in the post-disinfection period. Future study about the optimization of UVC-LED system targeting on this problem is demanded.

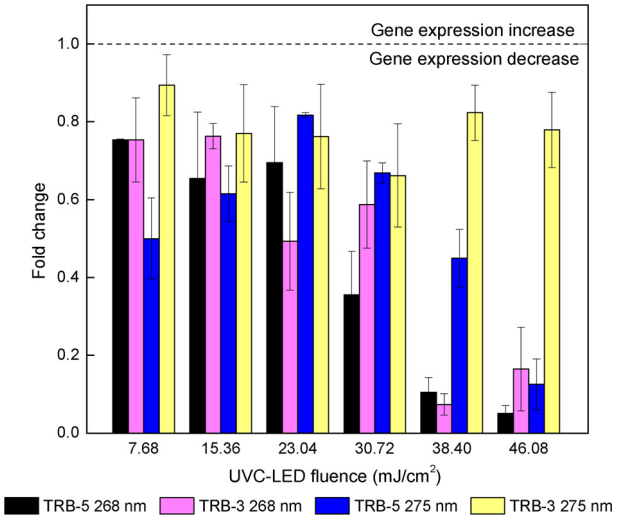

Fig. 5. Fold change expression of *tet*(L) in TRB after UVC-LED disinfection at 268 nm/275 nm and different fluences. Dashed line indicates no change referring to the gene expression level of the original bacteria.

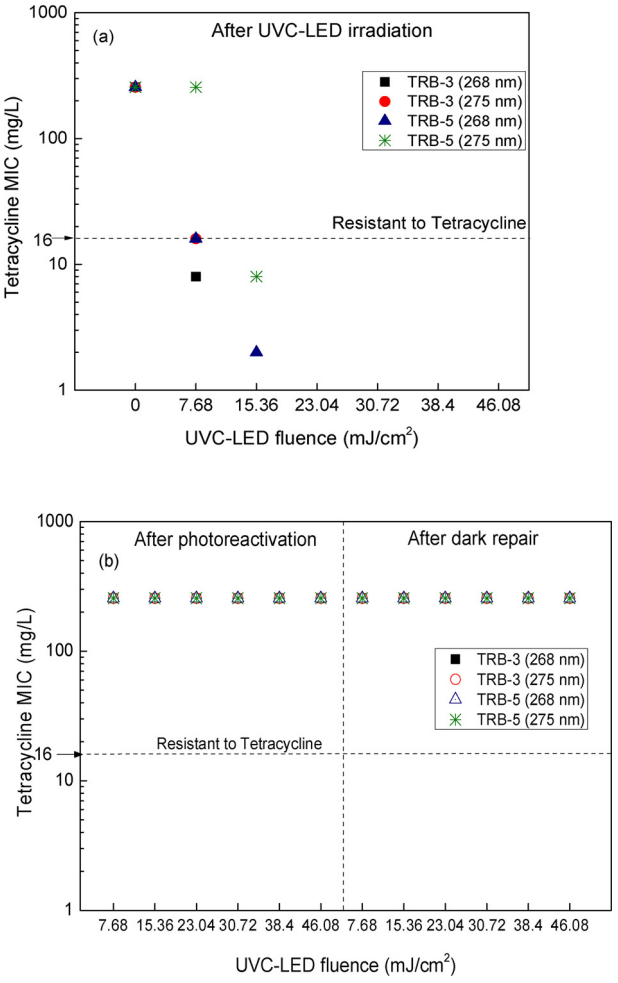

Fig. 6. Tetracycline MICs obtained by TRB-3 and TRB-5 (a) directly after UVC-LEDs disinfection and (b) after photoreactivation or dark repair, at 268 nm/275 nm and different fluences.

CRediT authorship contribution statement

Liang Shen: Conceptualization, Supervision, Writing - original draft. **Tiffany Maria Griffith:** Investigation, Writing - original draft. **Paul Onkundi Nyangaresi:** Investigation, Writing - review & editing. **Yi Qin:** Methodology. **Xin Pang:** Methodology. **Guolong Chen:** Resources. **Minglun Li:** Validation. **Yinghua Lu:** Funding acquisition. **Baoping Zhang:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.121968.

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