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Effects of single and combined UV-LEDs on inactivation and subsequent reactivation of *E. coli* in water disinfection

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ABSTRACT

Ultraviolet light emitting diodes (UV-LEDs) have shown a potential to replace traditional Ultraviolet (UV) pressure lamps for water disinfection. However, the research is not sufficient and hence, it is still difficult to make any logical conclusions. In this work, UV-LEDs with peak emissions at 267, 275, 310 nm and combined emissions at 267/275, 267/310 and 275/310 nm were applied to a batch water disinfection system. Under either single- or combined-wavelength situation, the inactivation efficiency, reactivation (due to photoreactivation and dark repair) after UV irradiation and electrical energy consumption were evaluated by way of the model bacterium *Escherichia coli*. It was found that, the 267 nm UV-LED had the highest inactivation efficiency than other UV-LEDs. Although reactivation occurred after 267, 275, 267/275 and 275/310 nm UV-LEDs' irradiations, it occurred to a lesser extent in dark repair than in photoreactivation, demonstrating that photo-effect is the dominant mechanism of reactivation. In addition, decay phase was more prominent than reactivation in dark repair. However, the irradiation by the 275 nm UV-LED showed a better persistence against reactivation which could be attributed to protein damage at 275 nm. No synergistic effect for combined wavelengths was observed in this study. The electrical energy consumption was lower for the 275 nm UV-LED than the other UV-LEDs which was attributed to its higher wall plug efficiency. This study showed the variation principle between the single and combined UVB/UVC-LEDs in inactivation efficiency, inhibition of reactivation, synergistic effect and electrical energy consumption in treatment of *E. coli*, which is useful for the reasonable exploitation of UV-LEDs in water disinfection systems.

1. Introduction

UV irradiation with wavelengths in the germicidal range (200–320 nm), is the latest method of modern water and wastewater treatment (Kowalski, 2009). Currently low pressure (LP) and medium pressure (MP) mercury lamp emitting monochromatic emission at a wavelength of 253.7 nm and polychromatic emission light at a broad range of wavelengths, 200–600 nm respectively, are widely employed as a UV source in drinking water and wastewater treatment plants (Bolton and Cotton, 2011). The efficacy and the doses needed using these mercury lamps are well established for various pathogens which include bacteria, protozoan parasites, and viruses. (Abbaszadegan et al., 1997).

The newly emerging ultraviolet light-emitting diode (UV-LED) is a potential alternative of traditional UV mercury lamps because of the advantages such as: diversity in wavelengths, environmental friendliness (no mercury), compactness, robustness, faster start-up time (excluding warm-up), potentially less energy consumption, longer lifetime, and the ability to turn on and off with high frequency (Würtele et al., 2011). In addition, UV-LED reactors can best be utilized in small scale, which is especially convenient in remote areas in view of cost (Crawford et al., 2005; Lui et al., 2014). Such UV-LEDs are wide band gap semiconductors composed mostly of gallium nitride (GaN) and aluminum gallium nitride (AlGaN). Although the wall plug efficiency (WPE) of UV mercury lamps (15–35%) is higher than that of UV-LEDs (1–3%), the latter is

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expected to be improved significantly, being similar to the case seen in visible LEDs (Harris et al., 2013).

For UV-LEDs to be applied as a practical option, several studies have examined their application in inactivation of pathogens for water disinfection. Several of these studies have reported that UV-LEDs at wavelength around 265 nm have a relatively higher inactivation of microorganisms than other wavelengths in the UVB and UVC (200–300 nm) range (Chatterley and Linden, 2010; Bowker et al., 2011; Chevremont et al., 2012a; Oguma et al., 2013; Beck et al., 2017; Rattanakul and Oguma, 2018; Li et al., 2017). The inactivation by the UVB and UVC radiation is effected through the formation of lesions in the genomic DNA of the organisms. The major DNA lesions induced by the UV light are cyclobutane pyrimidine dimers (CPDs), pyrimidine 6-4 pyrimidone photoproducts (6-4 PPs), and their Dewar isomers (Ravanat et al., 2001; Sinha and Häder, 2002; Cadet et al., 2005; Friedberg et al., 2006). The presence of these UV-induced lesions would inhibit the normal replication of DNA resulting in inactivation of the microorganisms.

However, some microorganisms, particularly bacteria, are capable of reactivating by repairing their damaged DNA after UV irradiation by mechanisms such as photoreactivation and excision repair (dark repair) (Friedberg et al., 1995; Harm, 1980). This will greatly decrease the final inactivation result and thereafter health risks of infection, when UV radiation is used for microbial disinfection in water. Photoreactivation is a process where microorganisms utilize light in the wavelength range of 330–480 nm to activate a photolyase enzyme, which binds specifically to the CPDs (CPD photolyase) or 6-4 PPs (6-4 photolyase) and directly monomerizes the cyclobutane ring of the pyr <> pyr and protects the genome from deleterious effects of UV radiation whereas excision repair (dark repair) is a multistep, where an abnormal or damaged base is removed by two major subpathways: (i) base excision repair (BER) and (ii) nucleotide excision repair (NER) (Rastogi et al., 2010).

Previous studies have indicated that, medium pressure (MP) UV lamps have the ability to repress photoreactivation of *Escherichia coli (E. coli)* (Oguma et al., 2004). It has been suggested that, the repressed photoreactivation is due to the irreversible oxidative damage to photolyase by MP UV (Quek and Hu, 2008). Another study also assumed that the MP UV irradiation resulted in less photoreactivation due to induced damage to proteins other than DNA itself (Kalisvaart, 2004). In addition, MP UV emission wavelengths of 220–300 nm was reported to reduce the subsequent photorepair of *E. coli* by causing a disorder with endogenous photolyase a DNA repair enzyme (Oguma et al., 2002). The DNA of most of the microorganisms is believed to have an absorption maximum between 260 and 270 nm (LeChevallier and Kwok-Keung, 2004; Gates, 1930). Meanwhile the proteins that are responsible for infection usually show absorption maximum between 275 and 280 nm which is caused by the absorbance of aromatic amino acids tryptophan, and tyrosine and cystine (i.e. of disulfide bonds) (Schmid, 2001). Therefore, repression of photoreactivation can be attributed to protein damage at a wavelength between 275 and 280 nm, though more studies should be conducted to confirm this hypothesis.

Since effectiveness of UV light for inactivating microorganisms is in the UV-B and UV-C ranges of the spectrum (200–310 nm), similar to MP UV lamp, repression of the reactivation by UV-LEDs is possible, which has also been reported in the recent study. Specifically, the 280 nm UV-LED was found able to significantly repress reactivation (Li et al., 2017). However, the UV-LEDs are characterized by diversity in wavelengths, whereas MP UV lamp emits a continuous and broad spectrum of germicidal wavelengths. Therefore, the combination of UV-LEDs of different wavelengths may be necessary to suppress effectively the reactivation. This raises the study on the inactivation effect using the combined UV-LEDs. The synergistic effect is normally determined by comparing the results of log inactivation by combined disinfection treatments and the results from the sum of log inactivation by individual treatments (Koivunen and Heinonen-Tanski, 2005). Till now, a synergistic effect after application of UV-LEDs disinfection systems has only been reported in quite few references (Chevremont et al., 2012a; Nakahashi et al., 2014; Green et al., 2018). Some studies even reported contrasting finding of no synergistic effect (Oguma et al., 2013; Beck et al., 2017; Li et al., 2017). Nevertheless, the effectiveness of both inactivation and reactivation in cases of the combined UV-LEDs is of great importance for water disinfection.

Electrical energy efficiency is another factor involved in making an economically reasonable decision when designing disinfection systems. It is characterized by a parameter known as electrical energy per order (E_{EO}) which has been previously used for interpreting collimated beam data to estimate electrical efficiencies of LP UV and MP UV lamps for large-scale treatment of chemical contaminants (Sharpless and Linden, 2005). Therefore, the same parameter of electrical energy per order (E_{EO}) can be applied in UV-LED disinfection systems to determine their electrical energy efficiency. However, up to date only a few studies have considered both inactivation and electrical energy efficiency. In one study, the inactivation and electrical energy efficiency of 260, 280 and 260/280 nm UV-LEDs were compared (Beck et al., 2017). For *E. coli* inactivation in particular, the results of 260 nm and 280 nm UV-LEDs were not statistically different, which contradicts the earlier findings that the relative peak of bactericidal effectiveness is between 260 and 270 nm (Gates, 1930). However, it was reported that, the electrical energy efficiency of the 280 nm UV-LED and the 260/280 nm UV-LED combination was less than the 260 nm UV-LED. In another study, the 265, 280 and 300 nm UV-LEDs were used in inactivation of *E. coli*, *Bacillus subtilis spores*, *Bacteriophage Qβ*, *Pseudomonas aeruginosa* and *Legionella pneumophila* (Rattanakul and Oguma, 2018). It was reported that, although the 265 nm UV-LED had higher inactivation efficiency, the 280 nm UV-LED showed the lowest energy consumption. The differences in the electrical energy consumption were attributed to difference in wall plug efficiency. Note that, the wall-plug efficiency (radiant efficiency) is the energy conversion efficiency with which the system converts electrical power into optical power. In other words, the wall-plug efficiency is defined as the ratio of the radiant flux (total optical output power of the device) to the input electrical power (Barnes, 2007). However, we think that, not only does the electrical energy consumption depend on wall plug efficiency, but also on the water factor and the sensitivity of the microorganism at a particular wavelength.

As shown above, the studies on application of UV-LEDs for water disinfection are insufficient and from the few published ones, discrepancies exist on important factors such as inactivation efficiency, repression of reactivation of microorganisms after UV irradiation, combined effect of different wavelengths and electrical energy efficiency. Therefore, in this study, these factors are evaluated, with special attention on the potential synergistic effect of combined UV-LED wavelengths. We believe that, the results from this work will provide additional and beneficial information for consideration in application of UV-LEDs for water disinfection systems.

2. Materials and methods

2.1. Culturing and enumeration of microorganisms

In this study, *E. coli* was chosen as a model microbe because the presence of *E. coli* itself is a good indication on the presence of pathogens and they are a good measure of overall water quality

(Jackson et al., 2001). A pure culture of *E. coli* strain CGMCC 1.3373 provided by China General Microbiological Culture Collection Centre (CGMCC) were incubated in a shaker incubator in Luria-Bertani (LB) broth at 37 °C and 200 rpm for 5–6 h until log phase was reached, determined by measurement of OD600. The cells were collected by centrifugation (10,000 rpm, 10 min, and 4 °C), washed twice with a sterilized saline solution (0.85%), and then suspended in sterilized saline solution at a concentration of approximately 10^6 CFU/mL, determined by standard plate count method and measurement of OD600 through the UV-1780 UV-VIS spectrophotometer (Shimadzu, Japan). Un-irradiated (N_0) and irradiated samples (N) were collected respectively at time zero and exposure times, t (s). For enumeration, both un-irradiated and irradiated (immediately after irradiation and after exposure to light and dark conditions) samples were serially diluted before using the plate-streaking technique. Volumes of 100 μL of the diluted samples were spread on LB agar plate in triplicate and incubated while inverted at 37 °C for 18–20 h. Plates yielding 1 to 300 colonies were considered for analysis. Deionized water was used for all experiments (e.g. cultivation, irradiation and enumeration) in this study.

2.2. UV-LED and fluence measurement

UV-LEDs with emissions at 265, 275 and 310 nm and optical power output of 1.8, 1.6 and 1.3 mW respectively at current of 20, 20, 20 mA achieved at voltages of 6.0, 3.9, and 6.0 V respectively (Great Bright Company, China) were used. The emission spectra (Fig. 1a) of the UV-LEDs were measured with Spectro 320 Optical Scanning Spectrometer and exhibited peak emission wavelengths at 267, 275 and 310 nm with full widths at half-maximum (FWHM) of 12 nm, 10.5 nm and 8.9 nm respectively. The UV-LEDs were soldered in single boards and fixed with pins to a circular board of 50 mm diameter to form a 24 mm square array consisting of three rows of three UV-LEDs. The batch reactor contained an array of nine single wavelength or a combination of 5 and 4 UV-LEDs each connected in series and fixed in a board, a magnetic stirrer, power source and a microbial sample in a petri dish (Fig. 1b). Irradiance was measured at the same water surface level using IL-1700 radiometer with SED 270 detector (International Light, USA). An equivalent irradiance of around 0.384 mW/cm^2 was used in all the UV-LEDs. To achieve this, current/voltage was varied using one Keithley power source (Fig. S1) for single wavelength and for two wavelength combinations, two Keithley power sources (Fig. S2) were used. The fluence was a product of irradiance and exposure time, t (s) (Bolton and Linden, 2003).

2.3. UV irradiations

All the UV irradiation were conducted inside a clean chamber with a fan that maintain the chamber temperature at 25.4 °C (Fig. S3). At the same time, the fan helped to maintain the temperature of the powered UV-LEDs at a temperature of 31.4 °C (Fig. S4) which was within the manufacturer's requirement.

2.3.1. Inactivation experiments

Before UV irradiation, 20 mL of microbial suspension was obtained from the suspended bacteria cells in the sterilized saline solution at a concentration of approximately 10^6 CFU/mL, 5 mL was taken away for bacterial count and the rest (15 mL) was placed in a petri dish with 60 mm diameter (6 mm water depth) for irradiation. For the UV-LEDs to reach stable emission stage, they were powered on for 5 min before irradiation. While being stirred with a sterile magnetic stir bar, the microbial suspension was irradiated at 2.2 cm from the UV-LED source. For changing the different samples, the UV-LEDs were powered off for less than 30 s which did not have a significant effect on the irradiance. UV irradiation was done for all single and combined wavelengths in this study. Samples were taken followed by steps of serial dilution, plating, incubation and enumeration.

2.3.2. Reactivation experiments

To investigate if the 275 nm has a repressive effect on microorganisms' reactivation after UV irradiation, the irradiation was done using the 267, 275, 267/275 and the 275/310 nm UV-LEDs at a fluence that led to 3.0 and 4.0-log inactivation. Same as in section 2.3.1, before UV irradiation, 20 mL of microbial suspension was prepared, 5 mL taken away for bacterial count and the rest (15 mL) was placed in a petri dish for irradiation. Immediately after irradiation, 5 mL of the bacterial sample was transferred into 5 mL glass tube for bacterial count and the rest was used to perform reactivation process. The reactivations were conducted at controlled room temperature since any change in temperature affects reactivation (Salcedo et al., 2007). Fluorescent lamp-15 W, Philips, with a peak emission at 395 nm was placed at a distance of 30 cm from the samples. The reactivation period was 9 h and bacteria samples were collected at interval of 1.5 h. Dark repair was carried out in a dark box for the same time period and temperature as the samples exposed to the light. Each reactivation experiment was conducted three times and both un-irradiated samples (N_0), samples immediately after irradiation (N) and after reactivation N_t were taken followed by steps of serial dilution, plating, incubation and enumeration.

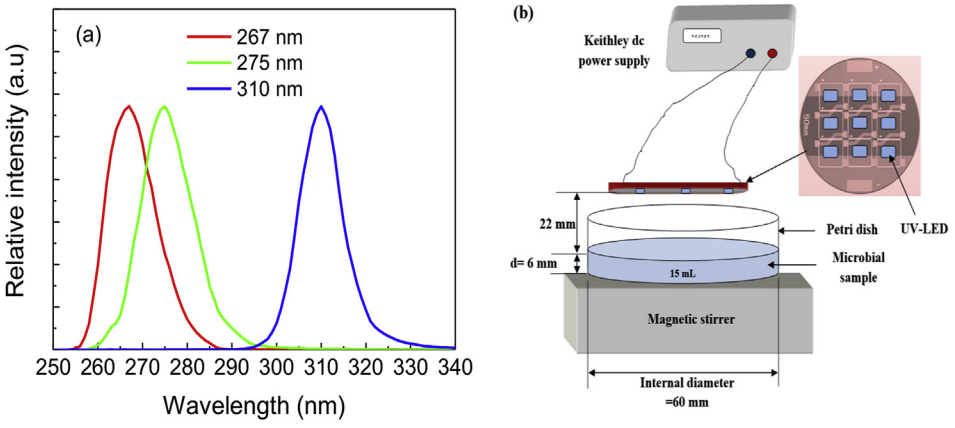

Fig. 1. (a) Emission spectra from the 267 nm, 275 nm, 310 nm UV-LEDs and (b) Set-up of a batch reactor.

2.4. Statistical analysis

2.4.1. Inactivation kinetics

The fluence-inactivation response curve of some microorganisms including *E. coli* has been observed to have a shoulder at low fluence, a log-linear phase at increasing fluence and a tailing phase at high fluence (Webb and Brown, 1976; Severin et al., 1983; Mossel et al., 1995; Cerf, 1997; Rattanakul and Oguma, 2018). In this study therefore, we used the shoulder model which does not consider the tailing phase (Eq. (1)) (Hijnen et al., 2006).

$$Log\left(\frac{N_0}{N}\right) = k \cdot Fluence - b \tag{1}$$

where N_0 and N is the number of colony (CFU/mL) before and immediately after UV irradiation, b is the y-intercept (a negative value since the curve is crossing the fluence axis at the UV fluence where log-linear relationship starts (offset).

2.4.2. Reactivation kinetics

2.4.2.1. Quantitative evaluation of reactivation. To evaluate the effect of reactivation, the percentage of reactivation either due to photoreactivation or dark repair was quantified using Eq. (2) (Lindenauer and Darby, 1994).

$$\text{Percentage of reactivation } (\%) = \frac{N_t - N}{N_0 - N} \cdot 100\,\% \tag{2}$$

where N_0 is the cell number before UV irradiation (CFU/mL), N is the immediate cell number after UV irradiation (CFU/mL), N_t is the cell number after reactivation for a period of time, t (CFU/mL).

In addition, the reactivation can be expressed as a function of the survival ratio (Eq. (3)) in respect of the initial microorganism concentration before the inactivation process (Kashimada et al., 1996).

$$S = \frac{N_t}{N_0} \cdot 100\,\% \tag{3}$$

where S is the survival ratio at time t (%); N0 and Nt have the same meaning as above.

2.4.2.2. Modelling photoreactivation. A non-linear regression model was used to model photoreactivation (Eq. (4)) (Nebot Sanz et al., 2007; Salcedo et al., 2007).

$$S = \frac{S_m}{1 + \left(\frac{S_m}{S_0} - 1\right) \cdot e^{-k_2 \cdot S_m \cdot t}} \tag{4}$$

where S_m is the maximum limit of the microorganisms' survival by reactivation and S_0 is the survival ratio immediately after UV irradiation, k_2 is the growth second-order reactivation rate constant.

Note that k_2 is not a pure reactivation rate constant, it is rather a model parameter that is adjusted to predict the experimental data whose physical meaning is related to the time required to reach S_m and then the stabilization phase (Nebot Sanz et al., 2007; Salcedo et al., 2007). Therefore, a pure reactivation rate constant, K (Eq. (5)) can be obtained from the derivatives of Eq. (4) and its maximum value (Eq. (6)) is obtained when S reaches half of S_m (Li et al., 2017).

$$K = \frac{ds}{dt} = k_2(S_m - S) \cdot S \tag{5}$$

$$K_{max} = \frac{k_2(S_m)^2}{4} \tag{6}$$

2.4.2.3. Modelling dark repair. A model that considers a low and brief reactivation period and a decay phase was used in modelling dark repair (7) (Nebot Sanz et al., 2007; Salcedo et al., 2007).

$$S = \frac{S_m}{1 + \left(\frac{S_m}{S_0} - 1\right) \cdot e^{-k_2 \cdot S_m \cdot t}} - M \cdot t \tag{7}$$

where M is the mortality, a zero-order decay rate constant, while the other parameters have the same meaning as in Eq. (4). Note that, S_m, k_2, k_r and M in Eqs. (4) and (5) have a clear physical significance.

2.5. Synergy of inactivation

Synergistic effect of combined wavelengths on microorganism inactivation was compared from the results of log inactivation by combined UV-LEDs and the results from the sum of log inactivation by individual UV-LEDs. Therefore, the synergy values can be calculated using Eq. (8) (Koivunen and Heinonen-Tanski, 2005).

$$\begin{aligned} Synergy\ (Log\ units) = {} & Log\ inactivation\ by\ combined\ UV - LEDs \\ & - Sum\ of\ \log\ inactivation\ by\ individual\ UV \\ & - LEDs \end{aligned} \tag{8}$$

2.6. Electrical energy determination

The electrical energy ($E_{E,\,N}$) for a specific N-log reduction of microorganisms can be determined using Eq. (9) (Beck et al., 2017).

$$E_{E,N} = \frac{A \cdot F_N}{3.6 \cdot 10^3 \cdot V \cdot C \cdot WF} \tag{9}$$

where $E_{E,\,N}$ is the electrical energy for a specific *N* -log reduction of each sample, (in kWh/m^3). A is the irradiant surface area (cm^2) and F_N is the fluence required for N-log reduction (mJ/cm^2). The value of 3.6×10^3 is a unit conversion constant for W and kW, s and h, mL and m^3, V is the volume of sample (mL). C is the wall plug efficiency calculated from data given by manufacturer using Eq. (10) (0.015, 0.021, 0.011, 0.019, 0.013 and 0.016 for 267, 275, 310, 267/275, 267/310, 275/310 nm UV-LEDs respectively) and *WF* is the water factor calculated using Eq. (11) (Bolton and Linden, 2003).

$$C = \frac{P_{output}}{P_{input}} = \frac{F_A}{I_A \cdot V_A} \tag{10}$$

where P_{output} is optical power (mW) of the UV-LEDs, P_{input} is the applied electrical power (mW), I_A is the applied current (mA), V_A is the applied voltage (V), and F_A is the radiant flux (mW).

$$WF = \frac{1 - 10^{-\alpha\iota}}{\alpha\iota \ln 10} \tag{11}$$

where α is decadic absorption coefficient (cm^{-1}) and ι is the vertical path length (cm) of the water in the Petri dish (the values of WF are shown in Table 1).

Note that, the decadic absorption coefficient (absorbance for a

1 cm path length) for the single wavelengths was determined using a 1 cm-diameter quartz cuvette and UV-1780 UV-VIS spectrophotometer (Shimadzu, Japan). The cuvette was filled with sterilized saline water solution that was used as a medium in the disinfection experiments and inserted in the UV-VIS spectrophotometer the absorbance values measurement. For the combined wavelengths, the absorbance was approximated as the average of the absorbance values of the respective combined wavelengths. Values of decadic absorption coefficient of different wavelengths are shown in are shown in Table 1.

3. Results and discussion

3.1. E. coli inactivation by UV-LEDs

Inactivation profile of *E. coli* by different UV-LED wavelengths is shown in Fig. 2. A shoulder and tailing, were observed in this study. The shoulder could have been due to fluence-dependent repair process and the threshold value represents the number of sites of an organism that must be destroyed before inactivation occurs (Webb and Brown, 1976; Severin et al., 1983; Mossel et al., 1995). Tailing on the other hand, may have resulted from individuals in a population cultured from a pure colony that was not identical with respect to disinfection or due to aggregation of microbial suspension that occurs during treatment (Cerf, 1997).

Inactivation rate was obtained from log-linear part on fluence-response curve. The 267 nm UV-LED had a higher log inactivation than the other wavelengths followed by the 267/275, 275 nm UV-LED and the 310 nm UV-LED was the least (Fig. 2). In addition, the 267 nm UV-LED had a higher k value of 0.42 (Fig. 3) which is similar to the reported value of 0.43 and 0.41 respectively in Refs. (Oguma et al., 2013; Li et al., 2017). The k value at 275 nm UV-LED was 0.292 which was lower than 0.422 reported in Ref. (Bowker et al., 2011). This may be due to different experimental set-up and use of different *E. coli* strain. However, k value of 0.292 at 275 nm in this work was similar that of 0.29 and 0.30 both at 280 nm in Ref. Oguma et al.'s work (2013) and Li et al. (2017) respectively. This is reasonable because the 275 nm is close to 280 nm. Note that, for the 267/275 nm UV-LED combined, $k = 0.391$ was greater than $k = 0.292$ for the 275 nm UV-LED. This could have been caused by the higher germicidal effect at 267 nm in the combined wavelength which is the relative peak of bactericidal effectiveness as earlier reported in literature (Gates, 1930). The k values of the other single and combined wavelengths were the lowest and any combination having the 267 nm UV-LED, its k value slightly increased showing that the 267 nm UV-LED had an influence on inactivation efficiency of the combined UV-LEDs. Though the k values were not used earlier (Green et al., 2018), the inactivation efficacy and performance of UV-LEDs emitting at 259, 268, 275, 289, and 370 nm were effectively compared against a low pressure mercury lamp at 253.7 nm for *E. coli*, *Listeria* and *Salmonella* pathogens using Log Count Reduction (LCR). At an equivalent UV dose (7 mJ cm^{-2}), the UV-LEDs emitting at 259 and 268 nm achieved the highest LCR out of the tested wavelengths which is in agreement with our results in the case of the 267 nm. In our work, the fluence required to attain above 3.0-log and 4.0-log inactivation was 8.78, 10.09, 12.26 mJ/cm^2 and 11.52, 15.35 and 23.04 mJ/cm^2 respectively for the 267, 267/275, 275 nm respectively while for the other UV-LEDs, they required a relatively higher fluence at the same log inactivation. This finding is in agreement with the previous studies (Chatterley and Linden, 2010; Bowker et al., 2011; Chevremont et al., 2012a; Oguma et al., 2013; Beck et al., 2017; Rattanakul and Oguma, 2018; Li et al., 2017). Compared to the other UV-LEDs, the 267 nm UV-LED required a lower fluence of 8.78 mJ/cm^2 to attain above 3.0-log inactivation for *E. coli*.

Table 1
Decadic absorption coefficient, α (cm^{-1}) and water factor, WF for different wavelengths.

Wavelength (nm)	α (cm^{-1})	WF
267	0.0103	0.9929
275	0.0096	0.9934
310	0.0034	0.9977
267/275	0.0100	0.9931
267/310	0.0069	0.9952
275/310	0.0065	0.9955

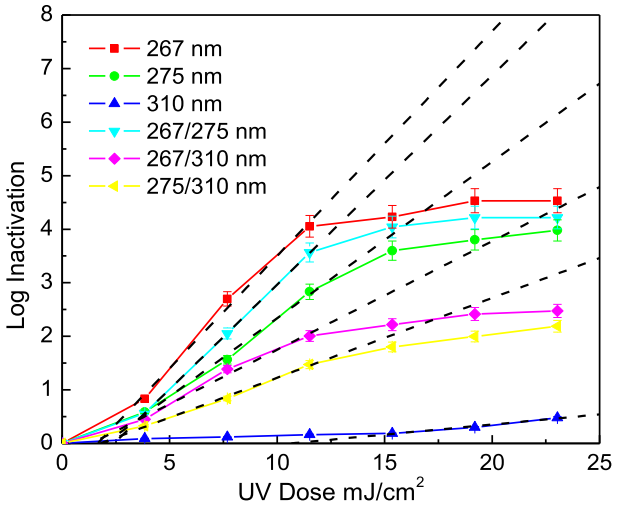

Fig. 2. Inactivation profile for *E. coli* under exposures of 267, 275, 310, 267/275, 267/310 and 275/310 nm UV-LEDs. Dashed lines indicate linear fit of log-linear part. Error bars represent standard deviation from 3 experimental data.

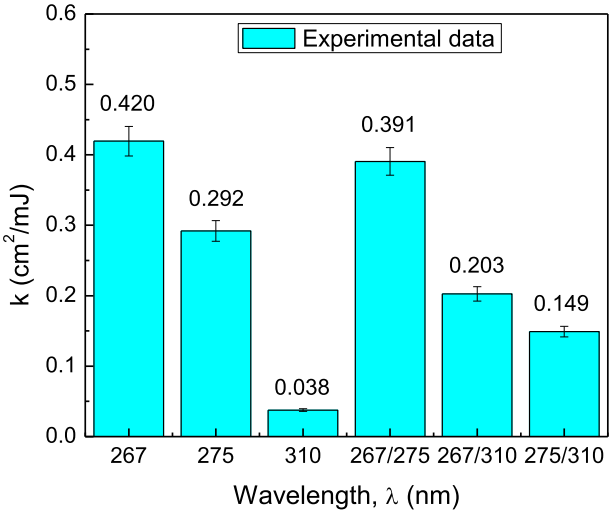

Fig. 3. Inactivation rate constants, k (cm^2/mJ) for *E. coli* at different single and combined UV-LED wavelengths obtained from log-linear part on fluence-response curve. Error bars represent standard deviation from 3 experimental data.

3.2. Photoreactivation

The photoreactivation percentages from the samples irradiated by the 275 nm UV-LED was statistically lower ($p < 0.005$) than the other UV-LEDs in this study in both the 3.0-log and 4.0-log

inactivation (Fig. 4). Note that, the irradiation by the 267 nm UV-LED had a higher inactivation efficiency than the 310/275 and 267/257 nm UV-LEDs' irradiation. However, the photoreactivation percentages after the 310/275 and 267/275 nm UV-LEDs' irradiation were statistically lower ($p < 0.05$) than those for the 267 nm UV-LEDs' irradiation (Fig. 4). This observation can be attributed to the presence of 275 nm in the 310/275 nm combination which helped to lower photoreactivation percentages. This finding is in agreement with previous study which reported that, *E. coli* photoreactivation was significantly repressed at 280 nm which is also close to 275 nm in our study (Li et al., 2017). In another previous study, adenovirus was effectively inactivated by the 285 nm UV-LED irradiation and the effective inactivation was attributed to proteins damage (Oguma et al., 2016). The mechanism of inactivation by the MP UV lamp which is capable of repressing *E. coli* reactivation after UV irradiation, may partly be attributed to damage to proteins which show absorption maximum between 275 and 280 nm (Schmid, 2001). By considering photoreactivation, the log inactivation decreased drastically as photoreactivation time increased. The decrease was lower for the 275 nm UV-LED leading to significantly higher log inactivation ($p < 0.005$) than the 267, 267/275 and 275/310 nm UV-LEDs in both 3.0-log and 4.0-log inactivation (Fig. 5a and b). Therefore, the repressive effect observed at 275 nm in this study may be attributed to protein damage, which is irreparable by the DNA repair mechanisms. The lower percentage of photoreactivation in both the UV-LEDs at a higher fluence (4.0-log inactivation) (Fig. 4b) as compared to lower fluence (3.0-log inactivation) (Fig. 4a) explains also the need of increasing fluence on minimizing photoreactivation which is in agreement with previous research studies (Lindenauer and Darby, 1994; Nebot Sanz et al., 2007).

The model described in Eq. (4) was applied to the photoreactivation experimental data using non-linear regression. A good fit of the model to the photoreactivation experimental data was observed (Fig. 6). The asymptotic shape of the curves was obtained which were consistent with a typical inactivation–reactivation curve (Nebot Sanz et al., 2007; Salcedo et al., 2007). Table 2 gives the values of the estimated kinetic parameters (S_m and k_2). Both S_m and k_2 showed a consistent behavior with the fluence that led to 3.0-log and 4.0-log inactivation. S_m showed a negative exponential tendency with the fluence which indicates that high fluence (4.0-log inactivation) produce severe damage on bacteria, making their reactivation low, while low fluence (3.0-log inactivation) allows the photoreactivation to take place. On the other hand, k_2 showed an inverse tendency in which it increased with the fluence. However, the increase in k_2 with the fluence is due to k_2 is not a pure reactivation rate constant as earlier explained and therefore K_{max} (Eq. (6)) was used to interpret the rate of reactivation. As in the case of S_m, K_{max} also showed a negative exponential tendency with the fluence indicating that, high fluence reduces K_{max}. The 275 nm UV-LED had the lowest S_m and $k_{r,max}$ as compared to the 267, 267/275 and 275/130 nm UV-LEDs in both the 3.0 and 4.0-log inactivation (Table 2). Note that, the low S_m and K_{max} indicates an extended induction phase and a short exponential phase (Fig. 6) and therefore, the 275 nm UV-LED could reduce both the S_m and K_{max} which means that, photoreactivation can be inhibited by UV-LEDs at 275 nm.

3.3. Dark repair

The lowest dark repair percentages were found in the irradiation by the 275 nm UV-LED than the 265, 275/310 and 265/275 nm UV-LEDs in both the 3.0-log and 4.0-log inactivation. The negative values of the dark repair percentages, indicates a decay phase where the colony count was below the initial count immediately before the dark conditions (Fig. 7). Although the dark repair percentages (below 0.02%) were much lower than photoreactivation percentages (above 0.13%), any bacteria survival can cause harmful effect to human being. The low dark repair percentages led to insignificant differences in log inactivation in both the 3.0 and the 4.0-log inactivation (Fig. 8). Both reactivation and a decay phase occurred in dark repair which was not the case in photoreactivation for a period of 9 h.

The dark repair survival curves showed a low and short reactivation period followed by a dominating decay phase. A good fit of the model (Eq. (7)) to the dark repair experimental data was obtained (Fig. 9a and b). This finding was in agreement with the previous studies in Refs. (Nebot Sanz et al., 2007; Salcedo et al., 2007). However, there was disagreement in the reports of the recent study, where only reactivation was reported during the dark repair (Li et al., 2017). According to our results, we think that, the slow reactivation and keeping the bacteria sample in absence of any source of food or light, can lead to a decay phase.

The values of the dark repair kinetic parameters- S_m, k_2, K_{max} were determined as earlier explained and M (mortality rate constant) was calculated experimentally from the slope of the linear end of the survival curves (between the 4.5 h and the final 9 h). The S_m and K_{max} values for the 275 nm UV-LED were lower than those

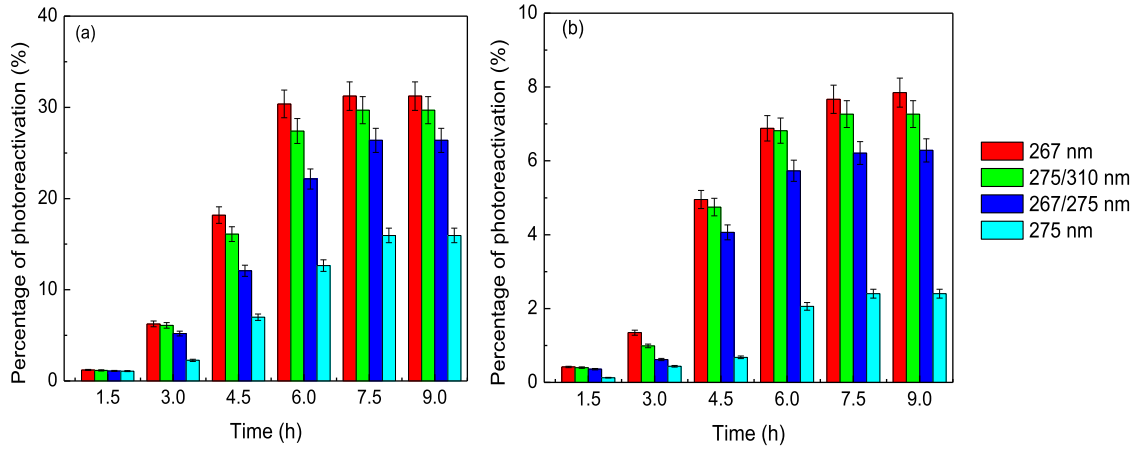

Fig. 4. Percentage of photoreactivation of *E. coli* for a period of 9 h after UV irradiation achieving (a) 3.0-log and (b) 4.0-log inactivation for different UV-LEDs. Error bars represent standard deviation from 3 experimental data.

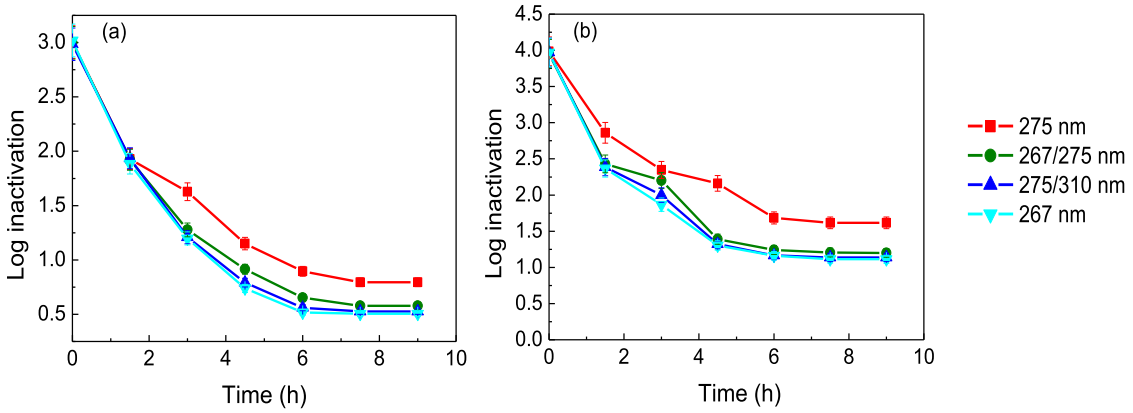

Fig. 5. Log inactivation when the photoreactivation for a period of 9 h was considered after UV irradiation for the fluence that led to (a) 3.0-log and (b) 4.0-log inactivation before photoreactivation. Error bars represent standard deviation from 3 experimental data.

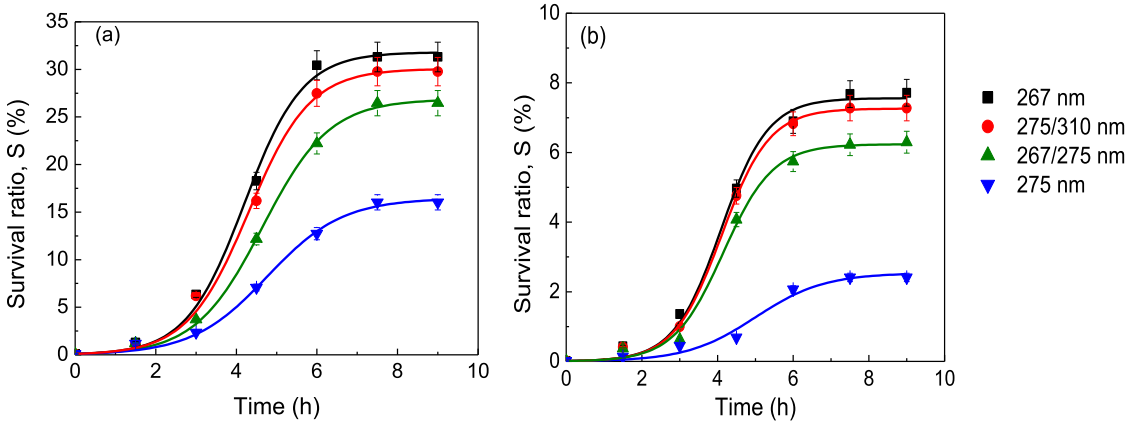

Fig. 6. Survival ratio due to photoreactivation as a function of time after UV irradiation achieving (a) 3.0-log inactivation and (b) 4.0-log inactivation for different UV-LEDs. Error bars represent standard deviation from 3 experimental data.

Table 2
Kinetic parameters of the logistic model applied to photoreactivation after UV irradiation achieving 3.0 and 4.0-log inactivation from the 267, 275 and 267/275 nm UV-LEDs.

UV-LED wavelength (nm)	S_m (%)		k_2 (% h^{-1})		R^2		K_{max} (% h^{-1})	
	3.0-log inactivation	4.0-log inactivation	3.0-log inactivation	4.0-log inactivation	3.0-log inactivation	4.0-log inactivation	3.0-log inactivation	4.0-log inactivation
267	31.80	7.56	0.043	0.21	0.997	0.998	10.87 ± 0.13	3.00 ± 0.08
275/310	30.06	7.26	0.044	0.22	0.998	0.999	9.94 ± 0.12	2.90 ± 0.07
267/275	26.86	6.24	0.045	0.24	0.999	0.997	8.12 ± 0.09	2.34 ± 0.05
275	16.45	2.54	0.065	0.43	0.998	0.988	4.40 ± 0.04	0.69 ± 0.01

for the 265, 275/310 and 265/275 nm UV-LEDs. In addition, the S_m and K_{max} values decreased with the increase in fluence which was a similar observation in photoreactivation (Table 3). However, the decrease of S_m and K_{max} in the dark repair was small of the order 10^{-1} and 10^{-2} as compared to that in photoreactivation of the order 10^{1} and 10^{0} respectively. This is an indication of a low and short reactivation in dark repair. The parameter M seems to be independent on both fluence and wavelength with average values between 1.2×10^{-3} - 2.8×10^{-3} (% survival/h). In addition, there is no significant change of M when either wavelength of fluence changes. On the other hand, the M values are very small, because, during the 4.5 h of reactivation a maximum survival of microorganisms of approximately 0.121 and 0.019% for the 3.0 and 4.0-log inactivation respectively will be produced. The mortality rate may be attributed to the residual effect of a radiation on the bacterial DNA and therefore will be independent on fluence and wavelength as observed in this study. The residual effect of radiation on the bacterial DNA occurs due to biochemical mechanism of actuation which needs some time to be manifested completely. Hence, it is also possible that, the mortality does not occur in photoreactivation experiments, because in this situation the repair of damaged DNA is more effective (Nebot Sanz et al., 2007). This explanation of possibility of absence of mortality in photoreactivation is in agreement with our finding. Therefore, though reactivation occurred after microorganisms' UV-irradiation, the occurrence was lesser in dark repair demonstrating that photo-effect is the dominant mechanism of reactivation. Therefore, our results demonstrated that, the 275 nm UV-LED showed better persistence against reactivation than the 265, 275/310 and 265/275 nm UV-LEDs which is may be attributable to protein damage at 275 nm.

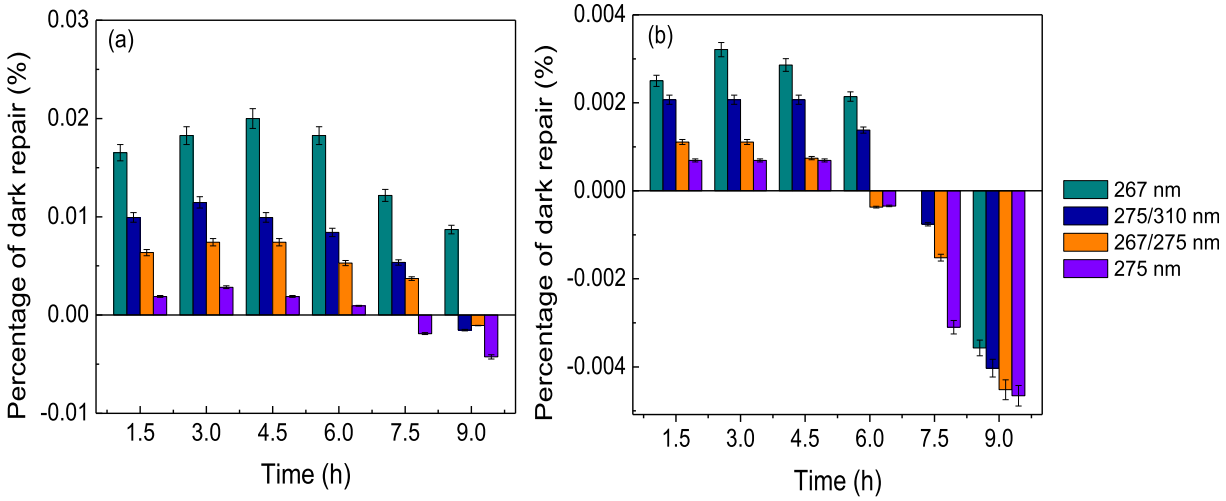

Fig. 7. Percentage of dark repair of *E. coli* for a period of 9 h after UV irradiation achieving (a) 3.0-log and (b) 4.0-log inactivation for different UV-LEDs. Error bars represent standard deviation from 3 experimental data.

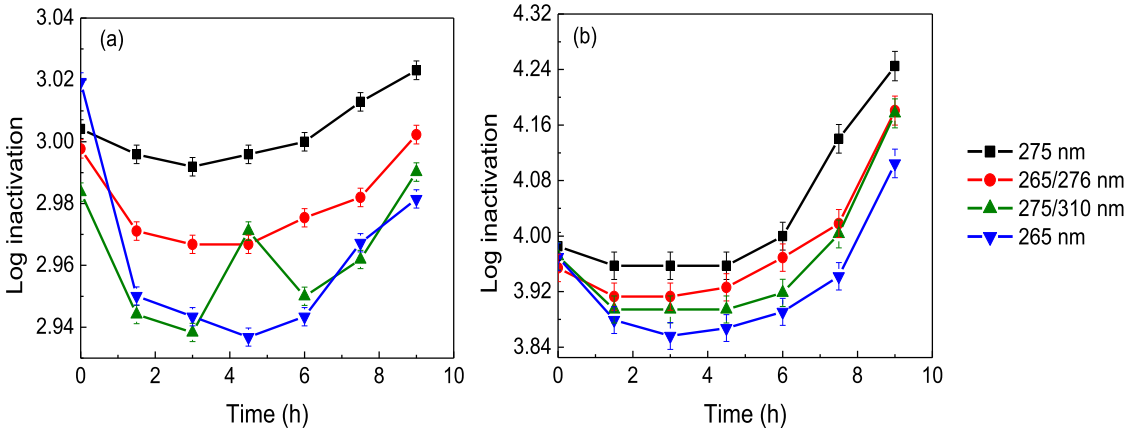

Fig. 8. Log inactivation as a function of time of dark repair after UV irradiation for 3.0-log and 4.0-log inactivation. Error bars represent standard deviation from 3 experimental data.

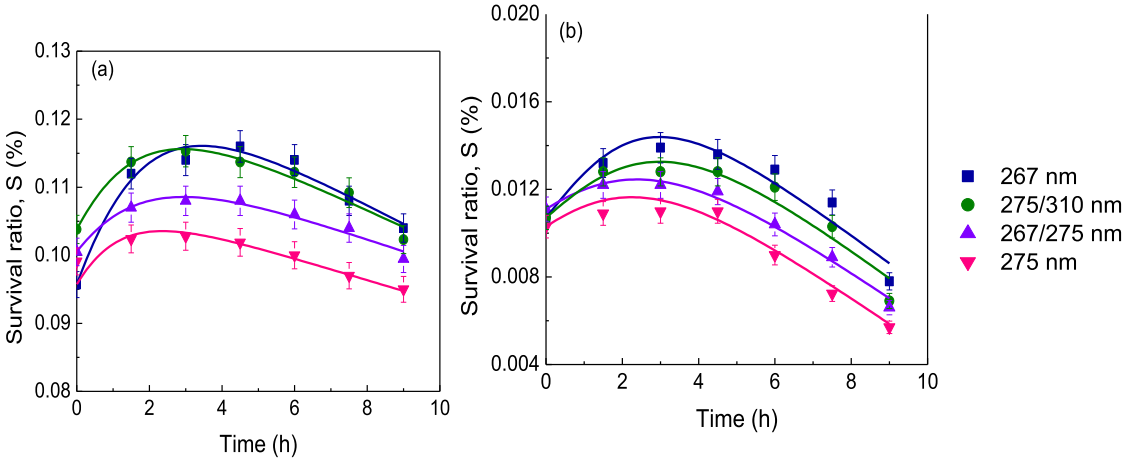

Fig. 9. Modelling of dark repair after UV irradiation achieving (a) 3.0-log inactivation and (b) 4.0-log inactivation from the 267, 275, 267/275 and 275/310 nm UV-LEDs. Error bars represent standard deviation from 3 experimental data.

Table 3
Kinetic parameters of the logistic model applied to dark repair after UV irradiation achieving 3.0 and 4.0-log inactivation from the 267, 275 and 267/275 nm UV-LEDs.

UV-LED wavelength (nm)	S_m (%)		k_2 (%)$^{-1}$		R^2		K_{max} (% h^{-1})		M (% h^{-1})	
	3.0-log inactivation	4.0-log inactivation	3.0-log inactivation	4.0-log inactivation	3.0-log inactivation	4.0-log inactivation	3.0-log inactivation	4.0-log inactivation	3.0-log inactivation	4.0-log inactivation
267	0.130	0.020	5.31	29.03	0.996	0.986	0.0224 ± 0.00019	0.0029 ± 0.00015	0.0028 ± 0.00035	0.0013 ± 0.00031
275/310	0.127	0.020	5.32	29.24	0.998	0.984	0.0215 ± 0.00018	0.0029 ± 0.00015	0.0025 ± 0.00058	0.0013 ± 0.00029
267/275	0.117	0.018	5.32	30.12	0.998	0.995	0.0182 ± 0.00017	0.0024 ± 0.00014	0.0018 ± 0.00029	0.0012 ± 0.00010
275	0.110	0.017	5.39	31.32	0.997	0.988	0.0163 ± 0.00016	0.0023 ± 0.00013	0.0016 ± 0.00010	0.0012 ± 0.00015

3.4. Effect of combining different wavelengths

Synergistic effect of 267/275, 267/310 and 275/310 nm UV-LED combinations was investigated. From observation, the sum of log inactivation by individual UV-LEDs that were weighted by their respective average irradiance percentages is equal to the log inactivation from those wavelengths combined (Fig. 10). The same observation was confirmed by using an independent two-tailed paired *t*-test i.e. the difference between the sum of the log inactivation proportions from individual UV-LEDs and the log inactivation from those wavelengths combined was not statistically significant ($p > 0.05$). This work indicates that there is no synergistic effect from 267/275, 267/310 and 275/310 nm UV-LEDs combinations in inactivating *E. coli*. Our finding is in agreement with previous studies (Oguma et al., 2013; Beck et al., 2017; Li et al., 2017) and also agreed with the second law of photochemistry (Stark-Einstein law) which states that, for each photon of light absorbed by a chemical system, only one molecule is activated for subsequent reaction. In other words, any photochemical effects of different wavelengths on a molecule should be independent of each other, achieving only as such inactivation as the sum of the photonic response from the individual wavelengths emitting separately. However, our study disagreed with other previous reports on presence of synergistic effect. In Green et al.'s study (2018), it was argued that synergistic effect following UV treatment at 259/289 nm was as a result of alternative inactivation mechanisms at 289 nm, leading to a hurdle effect. This may not be true because 289 nm is within the UVB/UVC wavelengths, whose mechanism of microorganism inactivation is similar (Ravanat et al., 2001; Sinha and Häder, 2002; Cadet et al., 2005; Friedberg et al., 2006). On

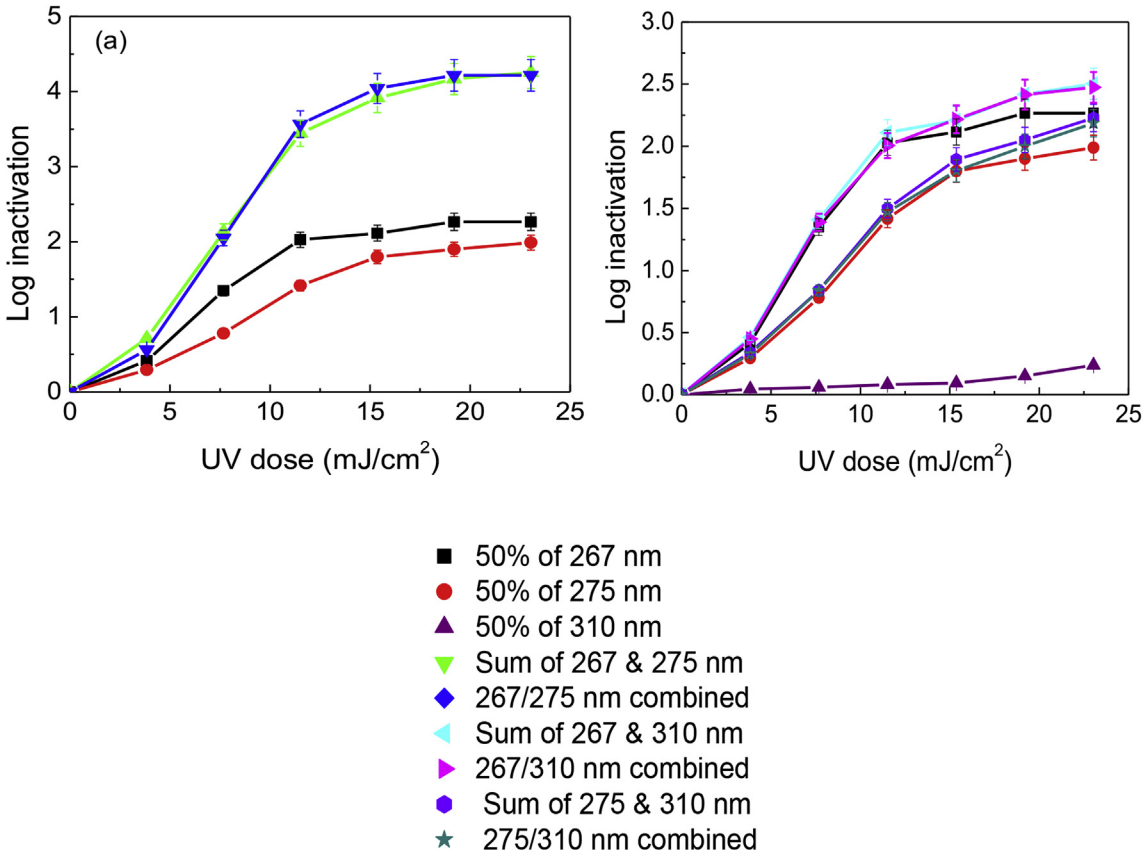

Fig. 10. Fluence response of (a) 267 and 275 nm, (b) 267, 275 and 310 nm UV-LED combinations compared with sum of their fluence response from separate LED exposures on *E. coli* inactivation. Error bars represent standard deviation from 3 experimental data.

the other hand, the synergistic effect was reported on UVC and UVA combination (Chevremont et al., 2012a; Nakahashi et al., 2014). In this case, we think that the synergistic effect may exist because, the mechanism of inactivation at UVA (320–400 nm) is believed to be different from that at UVC or UVB. Irradiation by UVA wavelengths is believed to cause lethal and mutagenic damage to an organism and in low doses it induces many physiological alterations, mainly the well-known radioinduced growth inhibition as reported in literature (Favre et al., 1985; Eisenstark, 1989). Therefore, more research should be performed at UVA/UVC with different microorganisms to confirm these previous reports.

3.5. Electrical energy efficiency

In any research involving pathogen inactivation, it is necessary to determine the electrical energy efficiency ($E_{E,N}$) of any UV source used. In our study the 267, 275, 310, 267/275, 267/310 and 267/310 nm UV-LEDs were used. The $E_{E,1}$ and $E_{E,2}$ were calculated using Eq. (9). The 275 nm UV-LED required lower electrical energy of 0.1367 and 0.2219 kWh/m^3 for $E_{E,1}$ and $E_{E,2}$ respectively in inactivation of *E. coli* as compared to $E_{E,1}$ and $E_{E,2}$ by the other UV-LEDs (Fig. 11). The lower $E_{E,N}$ of the 275 nm UV-LED can be attributable to the higher wall plug efficiency of 0.021 compared to that of other UV-LEDs. The 310 nm UV-LED in this study had higher $E_{E,1}$ of 1.7576 kWh/m^3. This is because, the germicidal effect is low at this wavelength as earlier explained and the wall plug efficiency of 0.011, could not have helped to lower its $E_{E,1}$. However, compared to the 310 nm UV-LED alone, a combination with 267 or 275 nm UV-LEDs led to lower $E_{E,1}$. This is because, the higher germicidal effect at the 267 and 275 nm helped in the lowering the $E_{E,N}$ in the combined wavelengths hence, suggesting that, the 310 nm UV-LED only, is not good but a combination with other LEDs is much better in lowering $E_{E,N}$. The results of our work agreed with previous study in Ref (Beck et al., 2017) where it was reported that, the electrical energy per order $E_{E,0}$ of 0.347 kWh/m^3 by the 280 nm UV-LED was slightly lower than 0.464 and 0.379 kWh/m^3 by the 260 and 260/280 nm UV-LEDs respectively. This is because, the wall plug efficiency of 0.005 by the 280 nm UV-LED was slightly higher than the wall plug efficiency of 0.004, and 0.00444 by the 260 nm and 260/280 nm UV-LEDs. Our study also agreed with another previous study in which the 280 nm UV-LEDs required lower $E_{E,3}$ of 0.17 as compared to 0.41 and 1.22 kWh/m^3 by the 265 and 300 nm UV-LEDs respectively (Rattanakul and Oguma, 2018). This is because, the 280 nm UV-LED had a higher wall plug efficiency of 0.019 as compared to 0.006 for the 265 nm UV-LED. The wall plug efficiency of 0.026 for the 300 nm UV-LED lowered its $E_{E,N}$ but not as much as the 275 nm UV-LED which is attributable to the lower germicidal effect of microorganisms at 310 nm as earlier explained. Our work has also shown that, not only does the electrical energy efficiency depend on the wall plug efficiency, but also on the vulnerability of microorganism at a given wavelength which determines the fluence F_N required to inactivate it as represented in Eq. (9).

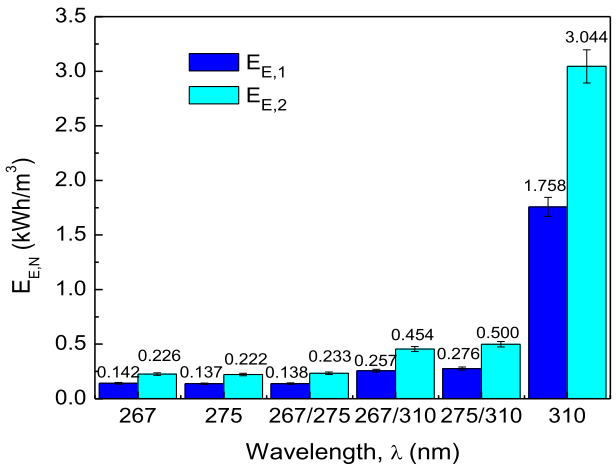

Fig. 11. Electrical energy per 1.0 ($E_{E,1}$) and 2.0 ($E_{E,2}$)-log inactivation of *E. coli* for different UV-LEDs. Error bars represent standard deviation from 3 experimental data.

4. Conclusions

Different UV-LEDs were used to study the inactivation of *E. coli* in a batch water disinfection system. The 267 nm UV-LED had the highest inactivation efficiency than other UV-LEDs. For the reactivation after irradiation, it was found that the dark repair was lower than photoreactivation, demonstrating that photo-effect is the dominant mechanism of reactivation. In dark repair, the decay phase was more prominent than reactivation. However, irradiation by the 275 nm UV-LED showed better persistence against reactivation. This was attributable to the higher possibility of damage to proteins at 275 nm. No synergistic effect for combined wavelengths was observed. The electrical energy consumption for the 275 nm UV-LED was lower than the other UV-LEDs in this study which is attributable to its higher wall plug efficiency. Compared with 310 nm only, however, a combination with 267 or 275 nm can significantly lower its electrical energy consumption. By considering repression of microorganisms' reactivation after UV irradiation and lower electrical energy consumption due to improved wall plug efficiency, the 275 nm UV-LED is concluded to be a better and a promising option in water disinfection. Note that, the electrical energy calculated only reflects the effect of specific wavelengths on specific microbe used in this work. In addition, the water used in this study did not contain any organic matter which may change the response. Therefore, detailed studies will be necessary with water containing organic matter, e.g. surface or lake water, and highly resistant pathogens to elucidate the practical application to tap and waste water treatment in both homes, hospitals and water treatment plants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2018.10.014.

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