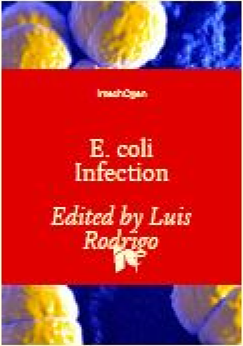
E. coli Infection



E. COLI INFECTION

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Chapter

Disinfection Efficiencies of UV-LED Irradiation on *E. coli* in Water

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Abstract

Ultraviolet light-emitting diode (UV-LED) is a newly emerging UV light source with a potential of replacing the conventional chemical methods, mercury UV lamps and xenon lamps in water disinfection applications. In this chapter, we will first give a general description on the status of *E. coli* disinfection in water by UV-LEDs. Then the main text will concentrate on our experimental studies. We will discuss the effects of single and combined UV-LED irradiation on *E. coli* in water, including the inactivation efficiency, the recover percentage after the UV-LED irradiation, the best wavelength for low energy consumption, differences in pulsed and continuous operations of UV-LEDs, effect of UVA-LED followed by UVC-LED irradiation and vice versa, and finally the effect of TiO_2 photo-catalyst.

Keywords: UV-LED, disinfection, photolytic, photocatalytic, simultaneous, sequential, synergistic effect, electrical energy efficiency

1. Introduction

Millions of people including children die every year from infectious diseases caused by various waterborne pathogens [1]. Among the pathogens, a group of bacteria called *Escherichia coli* (*E. coli*) is one of the known carrier of the diseases such as diarrhea, urinary tract infections, respiratory illness, pneumonia [2]. Since *E. coli* are typically found in the environment, foods and intestines of humans and animals, they have been widely used as fecal indicator bacteria in water quality analysis [3]. Numerous countries and world organizations put a limit count of zero per 100 ml *E. coli* for drinking water. Pass this limit, it is an indication of the presence of faecally related pathogens in water, and hence a potential risk of high level of microbial waterborne disease outbreak [4]. Therefore, different water disinfection methods have been employed in inactivation of the *E. coli* either in laboratory tests or in water disinfection plants. Among the different methods, conventional use of chemicals such as chlorine can lead to introduction of disinfectant-resistance to bacteria [5], change of water taste and production of odor [6] and harmful disinfection by-products (DBPs) such as trihalomethane (THM) compounds, and haloacetic acids (HAAs) that are carcinogenic, mutagenic and reproductive toxicants [7]. Ozone is reported as an effective alternative disinfectant to chlorine due to its ability of reducing microbiological challenge to downstream disinfection. However, the ozone is also known in forming by-products, particularly bromate [4], that can cause irreversible effects on humans such as renal failure and deafness [8]. The latest water disinfection method employs the use of ultraviolet (UV) light irradiation whose wavelength ranges from 200 to 400 nm. The UV light irradiation is currently attracting extensive attention in water and wastewater disinfection because of it is DBPs-free, and no need of chemicals that can cause ecological problems [9].

UV light is usually divided into four regions: vacuum (V) UV (100–200 nm), UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm) [10]. Note that, water and air absorb all wavelengths below 190 nm. Therefore, only the wavelengths between 190 and 380 nm can be used for biological effects [11]. Absorption of UV light by deoxyribonucleic acid (DNA)/ and ribonucleic acid (RNA) of a microorganism or virus inhibits its normal replication leading to cell death [12]. However, the UV damaged DNA of some microorganisms particularly *E. coli* bacterium is known to undergo repair by mechanisms such as photo-repair that requires light in the 300–500 nm wavelength range to activate a photolyase enzyme and dark repair that is light independent [13, 14]. This can greatly decrease the UV light disinfection efficiency hence posing a great chance of health risks of infection. The common UV light sources include: the sun, mercury pressure lamps, xenon lamps and newly emerging UV-light emitting diodes (UV-LEDs). Although the sun gives a cheap and green natural source of light, it is mostly unreliable and only UVA, and approximately 10% of UVB light reaches the earth's surface [15]. Mercury pressure lamps which exists in two types: low pressure (LP) and medium pressure (MP) mercury lamps emitting a monochromatic emission at a wavelength of 254 nm and polychromatic emission light at a broad range of wavelengths, 185–600 nm respectively [12], are the commonly used UV light sources in the current water disinfection systems [16]. However, these lamps are usually characterized with fixed wavelengths and limitations like short bulb lifetime, low energy efficiency, high operating temperatures and environmental pollution due to mercury [17]. On the other hand, xenon lamps are characterized by a broad range of wavelength (200–1100 nm), with 40% being UV consisting of UVC, UVB and UVA of about 20%, 8% and 12%, respectively [18]. Therefore, the xenon lamp can exhibit both photochemical effect due to the effect of the UV light, photophysical and photothermal effects due to its high intense pulses [19]. The three multi-target effects can lead to complete destruction of the cell wall and the nucleic acid structure of a microorganism [20]. In addition, the xenon lamps have high penetration, high energy conversion, no pre-heating is needed, faster start-up and no ozone generation [21]. Although the xenon lamp exhibits the above mentioned advantages over the sun and mercury pressure lamps, they have a high energy demand which is un-preferable especially in developing countries. The lamps are also limited in adjusting the duty rates and pulse frequency due to overheating hence affecting disinfection efficiency [22]. The newly emerging UV-LEDs are characterized with diversity in wavelengths within the UV range and have advantages such as environmental friendly (no mercury), compact and durable, faster start-up time, potential less energy consumption, longer lifetime, and a similar characteristic as xenon lamps of high frequency switching [23–25].

Therefore, due to their characteristics and advantages, the UV-LEDs have a high potential of replacing the other aforementioned UV light sources in water disinfection applications as demonstrated in literature [23, 26–28]. In addition, UV-LED reactors can best be utilized in small scale, which is convenient especially in remote areas since they can be photovoltaic powered [29–31]. Although the wall plug efficiency (WPE) of UV mercury lamps (15–35%) is higher than that of UV-LEDs ($<$10%), the latter is expected to be improved significantly, being similar to the case seen in visible LEDs whose WPE is currently around 80% [32, 33]. In water

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disinfection, the UV-LED irradiation can be applied in two modes: (i) pulsed light (PL) and (ii) continuous wave (CW) mode. Whereas PL irradiation is a fast non-thermal technology for decontamination based on the application of short pulses of high intensity of light [22], CW application on the other hand is based on the application of low light intensity [34]. Furthermore, the diverse nature of the UV-LED wavelengths allows for tailored irradiation in which the wavelengths can be irradiated at the same time (simultaneous) or one after the other (sequential). During the disinfection applications, the mechanism of the two irradiation modes can either be photolytic or photocatalytic. In photolytic disinfection, only UV light is involved such that the absorbed photons inactivate the pathogen in question [12]. Meanwhile, photocatalytic disinfection involves combining UV light and a photocatalyst such as TiO_2, that has the ability to absorb UV light of appropriate photon energy (Eq. (1)), and in an air-saturated or water environment, radicals such as OH• and $•O_2^-$ that are highly toxic towards microorganisms are produced [35]. Therefore, this chapter discusses effects of single and combined UV-LED irradiation on *E. coli* in water, including inactivation efficiency, recover percentage after the UV-LED irradiation, the best wavelength for low energy consumption, differences in PL and CW operations of UV-LEDs, effect of UVA- followed by UVC-LED irradiation and vice versa, and finally the effect of TiO_2 photo-catalyst.

$$E = h\frac{c}{\lambda} \tag{1}$$

AQ01 where E is the photon energy, h is the plank's constant =6.63×10^{-34}J s, c is the speed of light in a vacuum = 3.0×10^{8} m/s and λ is the wavelength of the UV light (m).

2. Indices of inactivation and repair performance for UV-LED disinfection

2.1 Evaluation of inactivation

2.1.1 Inactivation efficiencies

The inactivation efficiency of *E. coli* was analyzed by calculating log inactivation using Eq. (2).

$$\text{Log inactivation} = \text{Log}\left(\frac{N_0}{N}\right) \tag{2}$$

where N_0 and N are the colony count (CFU/mL) before and immediately after inactivation, respectively.

2.1.2 Synergistic inactivation efficiencies

Synergistic effect of combined wavelengths on the *E. coli* inactivation is 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 were calculated using the relation:

Synergy (Log units) = Log inactivation by combined UV-LEDs – Sum of log inactivation by individual UV-LEDs.

2.2 Evaluation of repair

2.2.1 Repair efficiencies

The percentage of repair either due to photo-repair or dark repair was quantified using Eq. (3) [36].

$$\text{Percentage of repair } (\%) = \frac{N_t - N}{N_0 - N} \cdot 100\% \tag{3}$$

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 repair for a period of time, t (CFU/mL).

In addition, the repair can be expressed as a function of the survival ratio (Eq. (4)) in respect of the initial microorganism concentration before the inactivation process [37].

$$S = \frac{N_t}{N_0} \cdot 100\% \tag{4}$$

where S is the survival ratio at time t (%); N_0 and N_t have the same meaning as above.

2.2.2 Repair kinetics

2.2.2.1 Modeling photo-repair

A non-linear regression model was used to model photo-repair (Eq. (5)) [38, 39].

$$S = \frac{S_m}{1 + \left(\frac{S_m}{S_0} - 1\right) \cdot e^{-k_2 \cdot S_m \cdot t}} \tag{5}$$

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

Note that k_2 is not a pure repair 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 [38, 39]. Therefore, a pure repair rate constant, K (Eq. (6)) can be obtained from the derivatives of Eq. (5) and its maximum value, K_{max} (Eq. (7)) is obtained when S reaches half of S_m [40].

$$K = \frac{ds}{dt} = k_2(S_m - S) \cdot S \tag{6}$$

$$K_{max} = \frac{k_2(S_m)^2}{4} \tag{7}$$

2.2.2.2 Modeling dark repair

A model that considers a low and brief repair period and a decay phase was used in modeling dark repair (Eq. (8)) [38, 39].

$$S = \frac{S_m}{1 + \left(\frac{S_m}{S_0} - 1\right) \cdot e^{-k_2 \cdot S_m \cdot t}} - M \cdot t \qquad (8)$$

where M is the mortality, a zero-order decay rate constant, while the other parameters have the same meaning as in Eq. (5). Note that, S, S_m, S_0, k_2, M and t in Eqs. (5) and (8) have a clear physical significance.

2.3 UV-LED technical parameters

2.3.1 Emission spectrum and optical power

The action spectrum of a microorganism is directly related to the LED emission spectrum i.e., wavelength and the FWHM [41–44]. Therefore, determination of LED emission spectrum before any experimental study is crucial. In this chapter, UV-LEDs with emissions at 265, 280, 310 and 365 nm, optical power of 1.8, 1.6, 1.3, 100 mW respectively at current of 20, 20, 20, 350 mA achieved at voltages of 6.0, 4.0, 6.0 and 4.0 V respectively (Great Bright Company, China) were used. The optical power was measured by an integrating sphere. Meanwhile the emission spectra measured with Spectro 320 Optical Scanning Spectrometer exhibited peak wavelengths at 267, 275, 310 and 370 nm with full widths at half-maximum of about 12, 10, 9 and 8 nm respectively (**Figure 1**).

2.3.2 Fluence measurement

The log inactivation of most pathogens is proportional to applied UV light fluence as given in Eq. (9), where k is the inactivation rate constant that varies from one microorganism to another.

$$\text{Log inactivation} = k \cdot \text{Fluence} \qquad (9)$$

Therefore, determination of fluence is critical for UV-LED disinfection applications. The common UV fluence determination methods include: Radiometry and chemical actinometry (iodide-iodate (KI) and ferrioxalate (FeO_x) actinometry). For UV-LEDs, fluence determination protocol employing the two methods for pathogen

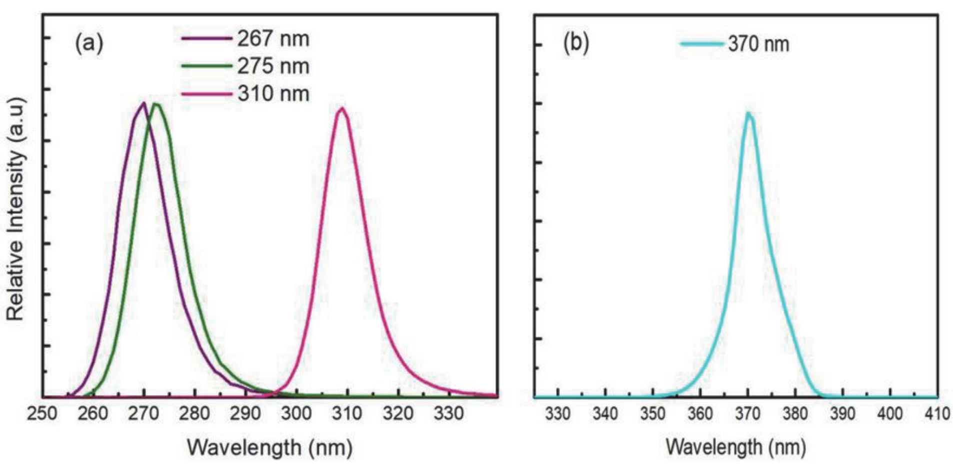

Figure 1.
AQ04 *Emission spectra from the (a) 267, 275, 310 and (b) 370 nm UV-LEDs.*

inactivation is well described in [45]. Therefore, this chapter employed radiometry only in the UV-LED fluence determination.

Average incident irradiance, $\overline{E_0}$ (mW/cm^2) was first determined at the same water surface level (l mm) from the UV-LEDs using IL-1700 radiometer with SED 270 detector (International Light, USA). Average fluence $\overline{F_0}$ (mJ/cm^2) inside the Petri dish was then calculated using Eq. (10) [45].

$$\overline{F_0} = \frac{\overline{E_0} \cdot PF \cdot DF \cdot WF \cdot RF \cdot t}{CF} \tag{10}$$

where PF the petri factor, DF the divergence factor calculated using Eq. (11), WF the water factor calculated using Eq. (12), RF the reflection factor taken to be 0.975 [46], t (s) the exposure time and CF is the collimation factor which was taken to be 1.

$$\mathrm{DF} = \frac{l}{l + D} \tag{11}$$

where l (cm) is the distance between microbial suspension surface and the UV-LED and D (cm) the microbial suspension depth (**Figure 2**).

$$\mathrm{WF} = \frac{\mathrm{I}_\lambda \cdot \left(1 - 10^{-\alpha_\lambda \cdot \mathrm{l}}\right)}{\mathrm{I} \cdot \alpha_\lambda \cdot \mathrm{l} \cdot \ln(10)} \tag{12}$$

where I (mW/cm^2) and I_λ (mW/cm^2/nm) are the total radiant power of the UV-LED and the radiant power at λ of the UV-LED, respectively, α_λ (cm^{-1}) is the decadic absorption coefficient of the microbial suspension at λ, and l (cm) is the microbial suspension depth. The decadic absorption coefficient (absorbance for a 1 cm path length).

2.3.3 Electrical energy determination

The electrical energy ($E_{E,N}$) for a specific N-log inactivation of microorganisms can be determined using Eq. (13).

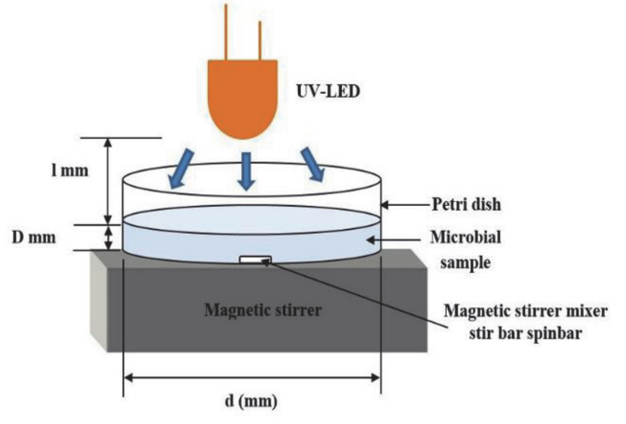

Figure 2.
Set-up of a batch disinfection reactor.

$$E_{E,N} = \frac{\pi\left(\frac{d}{2}\right)^2 \cdot F_N}{3.6 \cdot 10^3 \cdot V \cdot C \cdot WF} \tag{13}$$

where $E_{E,N}$ is the electrical energy for a specific N-log reduction of each sample, (in kWh/m^3), d (cm) the internal diameter of the Petri dish (**Figure 2**) and F_N is the fluence required for N-log inactivation (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 using Eq. (14) [47] and WF is the water factor calculated using Eq. (12) [46].

$$C = \frac{P_{output}}{P_{input}} = \frac{F_A}{I_A \cdot V_A} \tag{14}$$

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).

3. Disinfection efficiencies

The UV-LED disinfection efficiencies were discussed in four parts: (i) inactivation; (ii) repair; (iii) synergistic effect; and (iv) electrical energy efficiency.

3.1 *E. coli* inactivation efficiency

Comparative experiments with or without TiO_2 confirmed that, after 40 min of stirring in the dark, no inactivation occurred (data not shown). This indicates that UV light is the key requirement in both photolytic and photocatalyic inactivation. In both the photolytic and photocatalytic experiments, lower wavelengths were found to have a higher inactivation efficiency than longer wavelengths (267 > 275 > 310 > 370 nm) [48]. Specifically, in photolytic inactivation, an average fluence of 5, 7, 800 and 900 mJ/cm^2 was required by the 267, 275, 310 and 370 nm UV-LEDs, respectively per order log inactivation. Note that, a 4-log inactivation is required especially in Austria and Germany [12] in the inactivation of most microorganisms. Therefore, the 267 and 275 nm UV-LEDs required an average fluence of 12 and 15 mJ/cm^2, respectively for the 4-log to be achieved in *E. coli* inactivation. Meanwhile the other UV-LEDs required a relatively higher fluence for the same 4-log inactivation to be achieved [48, 49]. This finding indicated that UVC wavelengths have a higher germicidal effect in the inactivation of *E. coli* as also confirmed by their relatively higher average inactivation rate constant (k) of 0.4 and 0.3 for the 267 and 275 nm UV-LEDs, respectively compared to insignificant <0.03 for the 310 and 365 nm UV-LEDs. The finding was also consisted with the other studies in literature as reviewed in Ref. [50]. The DNA of most microorganisms is believed to have an absorption maximum of light between 260 and 270 nm [51], hence confirming the findings.

In photocatalytic disinfection, addition of TiO_2 (1.0 g/L) resulted to an interesting finding. Whereas the inactivation efficiency was increased in both the 310 and 370 nm UV-LEDs by the addition of TiO_2, that for the 267 and 275 nm UV-LEDs was drastically decreased [48]. Note that, anatase phase of TiO_2 that was used in our work has a bandgap of around 3.20 eV [52]. Therefore, in an air saturated or water environment, UV photon energy, $E \sim 5.12 \times 10^{-19}$ J is required to induce the generation of the reactive OH• radicals from the TiO_2 surface. The photon energy from the 267, 275, 310 and 370 UV-LEDs was calculated and found to be 7.45×10^{-19}, 6.87×10^{-19}, 6.42×10^{-19}, and 5.11×10^{-19} J, respectively. This indicates that, UVA wavelength is the most appropriate in photocatalytic disinfection as was confirmed by a significant enhanced inactivation efficiency by the 370 nm UV-LED when anatase phase of TiO_2 was added in the *E. coli* suspension [48]. The enhanced inactivation efficiency by the 370 nm UV-LED with TiO_2 is therefore attributable to the huddle effect of the UV photons and $OH^{\bullet}$ radicals. Other than their lower capability of radical production from the TiO_2 surface due to UV photon energy not within the optimum, the inactivation efficiency by the 267 and 275 nm UV-LEDs decreased with addition of the TiO_2 due to a screening effect by the TiO_2 which protected the *E. coli* against the strong UV photon of the UV-LEDs [53].

In another experiment, PL and CW UV-LED irradiation showed similar inactivation efficiency at equivalent average fluence [54]. Although similar inactivation efficiency was found, 267 nm UV-LED still had a slightly higher inactivation efficiency than the 275 nm UV-LED (**Figure 3**), which is in agreement with previous findings explained in the preceding paragraphs and also confirmed by reports in Ref. [51]. Although different UV-LEDs were employed, similar findings were also reported in other studies reported in literature [55–57]. However, an enhanced inactivation efficiency by PL over CW UV-LED irradiation is reported [58–61]. These discrepancies could be attributed mainly to unequal fluences between the PL and CW UV-LEDs, which is key in microbial inactivation. PL from xenon lamps is reported to cause enhanced inactivation efficiency than CW UV irradiation by mercury lamps [62]. The finding is due to xenon lamps' broad-spectrum UV content, short duration intense pulses and the high peak power which can lead to three multi-target mechanisms (photochemical, photophysical and photothermal) [63]. It should be noted that, the PL irradiation produced by xenon lamps is much different from that of the UV-LEDs in terms of emission spectrum, intensity, frequency switching. Therefore, the inactivation mechanisms of the PL xenon lamp may not apply to the UV-LEDs whose wavelengths are just within 200–400 nm and if a single UV-LED is used, almost a monochromatic wavelength is obtained compared with the broad range (200–1100 nm) from the xenon lamp. In addition, the current peak power of the UV-LEDs is still low (mW) which requires more improvements [64], compared to that of xenon lamps which is relatively high (kW) [65]. Unless the optical power is significantly improved, the *E. coli* inactivation efficiency by PL

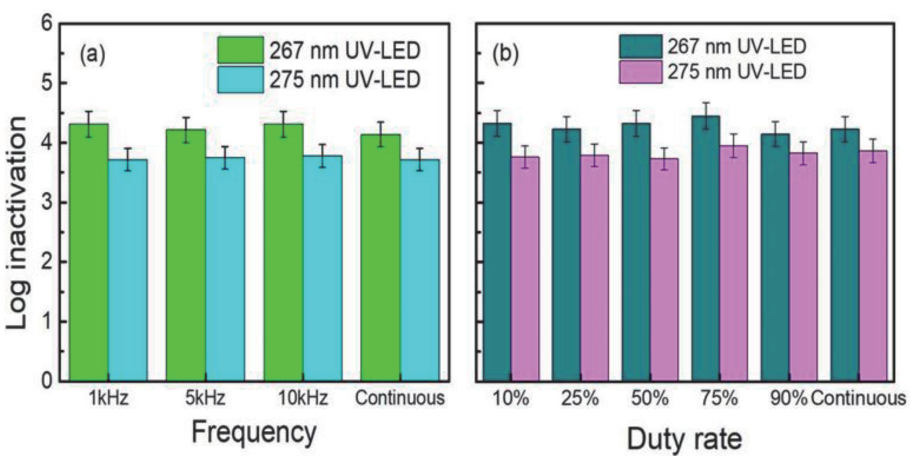

Figure 3.
Log inactivation at equivalent fluence of 17.3 mJ/cm² on E. coli *inactivation by the PL and CW UVC-LED irradiation after (a) varying frequency at 50% pulse rate and (b) varying duty rate at frequency = 1 kHz. Error bars represent standard deviation from triplicate experimental data.*

and CW UV-LED will still be equivalent. The only significant advantage of PL over CW UV-LED is its ability to suppress the heat generated during the UV-LED operation [54, 56]. This is due to the PL irradiation's ability to generate heat only during the short pulse and a cooling period can occur between each pulse. This ability was clearly observed when the PL showed a lower solder temperature as compared to the CW UV-LED at similar driving currents and ambient temperature (**Figure 4**).

Diversity of UV-LED wavelengths allows for tailored UV-LED irradiation involving 2 or more wavelengths either in simultaneous or sequential manner. Note that, irradiation by UVC and UVB wavelength is known in inducing lesion formation in the genomic DNA of a microorganism [66–68]. Meanwhile, irradiation by UVA causes formation of active substances such as reactive oxygen species that have lethal effects to a microorganism [69]. Due to their different inactivation mechanisms, this part of the chapter therefore concentrated only on simultaneous and sequential irradiation involving a combination of UVC(B) and UVA wavelengths. Note that, "UVC(B)" used here and henceforth in this chapter implies UVC or UVB. Compared to sum of corresponding single wavelength, simultaneous irradiation of 267, 275 or 310 with 370 nm UV-LED led to lower log inactivation values of 1.27, 1.23 and 0.64, respectively. Similarly, lower log inactivation of 0.92, 0.90 and 0.63 was also obtained in sequential irradiation of 267, 275 and 310 nm followed by the 370 nm UV-LED, respectively (**Figure 5**). These results indicate that the 370 nm UV-LED irradiation could have functioned in repairing the already UV damaged DNA, rather than damaging it [70, 71]. This assumption could be possible since the 370 nm is within the range of photo-repair light, 300–480 nm [13, 14]. On the other hand, higher log inactivation of 2.15 and 2.13 were achieved in sequential irradiation of 370 nm followed by 267 or 275 nm UV-LEDs, respectively. This log inactivation was also higher than that from the sum of corresponding single wavelength UV-LED irradiations, except for sequential irradiation of 370 nm followed by 310 nm UV-LEDs which achieved 0.98 log inactivation (**Figure 5**). Although the 370 nm (UVA) radiation can repair an already UV damaged DNA, the radiation on the other hand has an adverse effect when irradiated on un UV damaged DNA [72]. This phenomenon is known as concomitant photo-repair phenomenon in which inactivating light itself has the potential to photo-repair the UV-injured DNA [66].

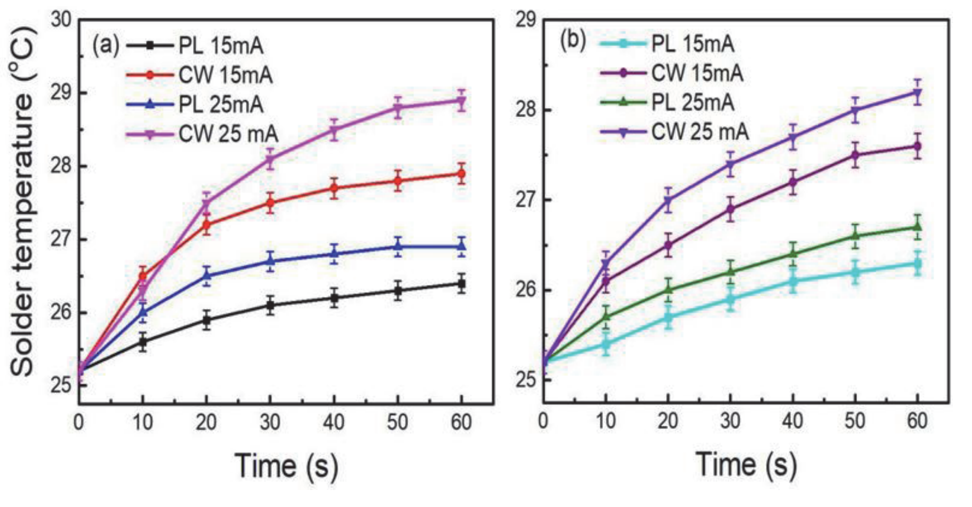

Figure 4.
Solder temperature as a function of operation period of the UVC-LEDs when operating in PL and CW mode; an ambient temperature of ~25°C, 50% duty rate at a frequency = 1 kHz for 267 nm (a) and 275 nm (b). Error bars represent standard deviation from triplicate experimental data.

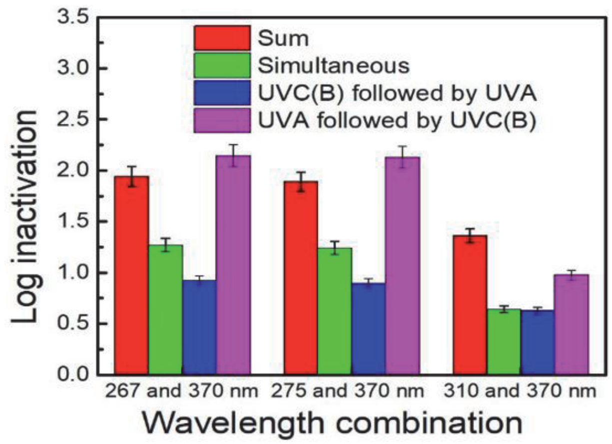

Figure 5.
E. coli *inactivation by combined wavelengths from different UV-LEDs. The 267, 275, 310 and 370 nm UV-LEDs provided an average fluence of 2.6, 2.6, 511.3 and 539.6 mJ/cm², respectively. Error bars represent standard deviation from triplicate experimental data.*

Note also that, the 310 nm could have a concomitant photo-repair phenomenon similar to the 370 nm wavelength. The 310 nm is within UVB band which is also known to produce lesions that damage microorganism DNA. In addition, the 310 nm is within the photo-repair light (300–480 nm), hence explaining this finding. These findings are consistent with the other studies in literature [71, 73].

3.2 *E. coli* repair efficiency

As mentioned earlier in the introduction section, *E. coli* has the ability to undergo repair after damage from UV light irradiation. In all the experiments conducted, photo-repair was more dominant with an average of above 5% of photo-repair and negligible or no dark repair occurred [48, 49], demonstrating that photo-effect is the dominant mechanism of *E. coli* repair. The dominance of photo-effect in *E. coli* repair was also reported in other studies in Refs. [40, 74, 75]. Considering the 267, 275 nm UV-LED, the same observation was confirmed by highest rate of photo-repair constant, $K_{max} > 4\%\ h^{-1}$ compared to that of dark repair, $K_{max} < 0.02\%\ h^{-1}$ [49]. By analyzing photo-repair after photolytic inactivation, 275 and 370 nm wavelengths were found to be appropriate in suppressing the photo-repair. In addition, when the same wavelengths were applied such that 275 nm followed after the 370 nm UV-LED irradiation, a much lower % of photo-repair is obtained compared to simultaneous, and 275 nm followed by 370 nm UV-LED irradiation. This observation is attributed mostly to damage of *E. coli*'s membrane at 370 nm [76], and both DNA and proteins at 275 nm [77]. Note that, no significant difference was observed in % of photo-repair for PL and CW UV-LED irradiation [54]. However, addition of TiO_2 led to an insignificant % of *E. coli* photo-repair (<1%) and for dark repair, mortality was registered [48]. The observation is attributed to the concomitant effect of the photons from the UV-LEDs and the $OH^{\bullet}$ radicals generated from the surface of UV irradiated TiO_2 that led to more damage to the *E. coli*. In addition, the mortality in the dark repair is attributed to a residual disinfecting effect of the $OH^{\bullet}$ [78].

3.3 Synergistic effect

During the *E. coli* inactivation, different wavelengths were combined and their synergistic effect evaluated. The irradiations were performed in both simultaneous and sequential manner. From the results obtained, simultaneous irradiation involving 267/275, 267/310 and 275/310 wavelength combinations from the UV-LEDs yielded absence of synergy in *E. coli* inactivation [49]. Note that, the 267 and 275 nm belong in the UVC band, meanwhile the 310 nm belong in the UVB band of UV wavelengths. The UVC and UVB wavelengths have similar inactivation mechanism [66–68], explaining the absence of synergy in this case. Although UVC(B) and UVA wavelengths are reported to have different disinfection mechanisms as highlighted in the introduction section, interesting findings were found both in simultaneous and sequential irradiation on *E. coli* inactivation. Simultaneous irradiation of 267, 275, 310 nm and their combination with 370 nm UV-LED led to lower log inactivation compared to the sum of log inactivation of the corresponding single wavelengths. Similarly, lower log inactivation was achieved for 267, 275 and 310 nm followed by 370 nm UV-LED irradiation (**Figure 6**). These findings highlighted the concomitant photo-repair phenomenon of the 370 nm UV-LED. It should be noted that, the 370 nm is within the range of photo-repair light (300–500 nm). Therefore, other than damaging the *E. coli* bacteria, the 370 nm light could have performed the role of photo-repair as also discussed in Refs. [70, 71]. Contrary, synergistic effect was found for 370 nm followed by 267 or 275 nm UV-LED. However, no synergy was found for 370 nm followed by 310 nm UV-LED irradiation (**Figure 6**). Due to the 370 nm light's ability to cause membrane damage to un UV damaged bacteria, when irradiated first then followed by the UVC wavelengths, more damage was realized which led to the found synergy. However, irradiating 310 nm UV-LED after the 370 nm could have resulted to the repair of the *E. coli* since the 310 nm UV-LE is within the photo-repair light, hence absence of synergy in that case.

3.4 Electrical energy efficiency

To make a viable decision in choosing an appropriate UV-LED to be applied in disinfection applications, it is necessary to determine the electrical energy efficiency ($E_{E,N}$) of the UV-LEDs for microorganism inactivation in water. For combined wavelengths, sequential irradiation involving UVA followed by UVC-LED showed higher inactivation and repair efficiencies of *E. coli* compared to the other combinations. Therefore, the electrical energy efficiency per order of magnitude ($E_{E,0}$)

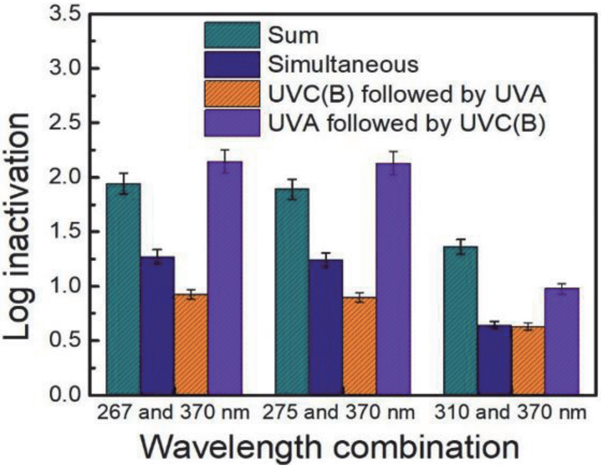

Figure 6.
Synergy from the combined UV-LEDs. The 267, 275, 310 and 370 nm UV-LEDs provided an average fluence of 2.6, 2.6, 511.3 and 539.6 mJ/cm², respectively. Error bars represent standard deviation from 3 experimental data.

was determined only for single wavelength irradiation (in both photolytic and photocatalytic) and UVA followed by UVC(B)-LED irradiation on the *E. coli* in water (**Table 1**). In both photolytic and photocatalytic disinfection, the 275 nm UV-LED required lower $E_{E,O}$. Although the addition of TiO_2 to the *E. coli* suspension led to an increase in the $E_{E,O}$ for the 267 and 275 nm UV-LEDs, that for the 310 and 370 nm UV-LEDs decreased. Meanwhile, for combined wavelengths, the 370 nm followed by 275 nm UV-LED irradiation required lower $E_{E,O}$ compared to the other combinations. The lower $E_{E,O}$ for the 275 nm UV-LED, and 370 nm followed by 275 nm UV-LED irradiation is attributed majorly to the higher wall plug efficiencies of the two UV-LEDs compared to the others [48, 49], a similar finding that is also reported in Ref. [79]. Note that, the decrease in $E_{E,O}$ for mostly the 370 nm UV-LED in photocatalytic disinfection is attributed two things: (i) its higher wall plug efficiency; and (ii) its photon energy being within the required to induce radicals on TiO_2 surface.

4. Conclusions

In this chapter, a general description on the status of *E. coli* disinfection in water by UV-LEDs has been highlighted. The main text concentrated more on our experimental studies in which the effects of single and combined UV-LED irradiation on *E. coli* in water, including the inactivation efficiency, the recover percentage after the UV-LED irradiation, the best wavelength for low energy consumption, differences in pulsed and continuous operations of UV-LEDs, effect of UVA-LED followed by UVC-LED irradiation and vice versa, and finally the effect of TiO_2 photo-catalyst, were discussed. Whereas the 267 nm UV-LED showed higher inactivation efficiency, the 275 nm UV-LED had competitive inactivation efficiency, higher repressive ability on *E. coli* repair and higher electrical energy efficiency. For photocatalytic disinfection, the 370 nm UV-LED was the most

Mode of irradiation	Photolytic/photocatalytic inactivation	UV-LED wavelength (nm)	$E_{E,O}$ (kWh/m³)
Single wavelength	Photolytic	267	0.4
		275	0.3
		310	17.2
		365	4.0
	Photocatalytic	267	0.6
		275	0.4
		310	16.0
		365	2.0
Combined wavelength (UVA followed by UVC or UVB	Photolytic	370 followed by 267	0.7
		370 followed by 275	0.5
		370 followed by 310	1.7

Table 1.
Average values of $E_{E,O}$ for different wavelength irradiations in E. coli *inactivation in water.*

appropriate. Although PL UV-LED was found to be effective in suppressing temperature rising CW operation, the two modes showed insignificant difference in *E. coli* inactivation and repair efficiency. For combined wavelengths, UVA (370 nm) followed by UVC-LED (275 nm) irradiation was effective in both inactivation, repair and electrical energy efficiencies.

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Conflict of interest

None.

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