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Comparison of UV-LED photolytic and UV-LED/TiO_2 photocatalytic disinfection for *Escherichia coli* in water

Paul Onkundi Nyangaresi[a], Yi Qin[a], Guolong Chen[b], Baoping Zhang[a,*], Yinghua Lu[c], Liang Shen[c,*]

[a] Department of Electronic Engineering, Laboratory of Micro/Nano-Optoelectronics, Xiamen University, Xiamen, Fujian, 361005, China
[b] Department of Electronic Science, Fujian Engineering Research Center for Solid-State Lighting, Xiamen University, Xiamen, 361005, China
[c] Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, Fujian, 361005, China

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ABSTRACT

Ultraviolet (UV) light irradiation is the latest disinfection method and TiO_2 is a well-known photocatalyst to generate reactive oxygen species. With the newly emerging UV-LEDs, however, the study of UV-LED/TiO_2 photocatalytic disinfection is still rear. In this work, by using *Escherichia coli* (*E. coli*) as a model microbe, UV-LED photolytic and UV-LED/TiO_2 photocatalytic disinfections were conducted under diverse conditions to examine their inactivation efficiency, repression potential on the repair of bacteria after UV inactivation and the electrical energy consumption. The results showed that increasing the irradiance enhanced the inactivation of *E. coli* in both photolysis and photocatalysis, especially the former. For a given irradiance of 0.49 mW/cm^2 from the 365 nm UV-LEDs, 1.0 g/L was found to be the optimal TiO_2 concentration. Then, the dependence of disinfection on wavelengths (265, 275, 310 and 365 nm) was studied at an irradiance of 0.49 mW/cm^2 with 1.0 g/L TiO_2. The highest inactivation efficiency was achieved by the 265 nm followed by the 275 nm UV-LEDs in both photolytic and photocatalytic disinfection. For these two wavelengths, the addition of 1.0 g/L TiO_2 can significantly repress the *E. coli* repair, whereas the inactivation efficiency becomes slightly worse. On the other hand, when irradiated by wavelengths of 310 and 365 nm, both inactivation efficiency and suppression of repair were significantly improved. Either with TiO_2 or not, the irradiation by 275 nm exhibited higher inactivation efficiency. Taking into full consideration of inactivation efficiency, suppression of repair and power consumption, the 275 nm UV-LED/TiO_2 was suggested to be a promising option for water disinfection.

1. Introduction

The safety of drinking water is still a serious worldwide issue because contaminated water can lead to waterborne diseases, such as typhoid fever (enteric fever), rotavirus diarrhea, and the pandemic disease cholera which currently, or historically, has been a leading cause of human death [1,2]. Therefore, the development of novel effective water treatment technologies is of great significance for human health. Among the emerging ones, photocatalytic disinfection has attracted much attention in the killing of bacteria, protozoa, virus fungus and algae [3,4], as well as degradation of refractory chemical pollutants in the source of drinking water [5].

Since 1970s, TiO_2 has been developed to be the most widely used catalyst in photocatalytic disinfection [4,6,7]. When exposed to light energy equal to or greater than its band gap ($\lambda < 385$ nm), the TiO_2 particles in an air-saturated or water environment can induce the generation of holes (h^+) and hydroxyl ($OH^\bullet$) in the valence band, and electrons (e^-) and superoxide ions ($\bullet O_2^-$) in the conduction band (Eq. (1)) [8,9]. In solution, the $\bullet O_2^-$ and $OH^\bullet$ can react to give H_2O_2, further $OH^\bullet$, OH^{-1} and $^1O_2$ (Eqs. (2) and (3)). Once both the $OH^\bullet$ and $\bullet O_2^-$ radicals have been produced on the surface of TiO_2, they interact immediately with the outer surface of an organism unless the TiO_2 particle has penetrated into the cell. Compared to the $\bullet O_2^-$ that are long-lived and cannot penetrate the cell membrane, the OH• radicals are short-lived, highly toxic towards microorganisms, very reactive in the oxidation of organic substances and their diffusion from the TiO_2 surface into the bulk solution is minimal. Therefore, $OH^\bullet$ radicals are the key species in the photocatalytic oxidation [9–13].

$$UV\ light \xrightarrow{TiO_2} e^- + h^+ \xrightarrow{O_2,\ OH^-,\ H_2O} \bullet O_2^- + OH^\bullet \quad (1)$$

in which e^- and h^+ denote the photogenerated conduction-band electrons and valence-band holes.

$$OH^\bullet + OH^\bullet \rightarrow H_2O_2 \quad (2)$$

* Corresponding authors.
E-mail addresses: bzhang@xmu.edu.cn (B. Zhang), shenliang@xmu.edu.cn (L. Shen).

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$$\bullet O_2^- + H_2O_2 \rightarrow OH^\bullet + OH^{-1} + 1\,O_2 \quad (3)$$

The remarkable advantage of TiO_2 photocatalytic disinfection (UV/TiO_2) results from the huddle effect provided by the photon energy from the UV light and the chemical energy from the $OH^\bullet$ radicals generated from the surface of the UV irradiated TiO_2 [14,15]. The UV light is known to induce lesions such as *cis-syn* cyclobutane pyrimidine dimers (CPD), (6–4) photoproducts and Dewar isomers, which causes inhibition of the normal replication of DNA and also bacterial mutations [16–18]. Meanwhile, the $OH^\bullet$ radicals are highly toxic towards microorganisms and their action on the bacterial cell membrane leads to the perturbation of different cellular processes and finally to bacterial death [19]. Therefore, for UV/TiO_2 disinfection, the wavelength of UV light turns out to be a critical parameter that should not only satisfy the UV irradiation but also match the energy gap of the photoactive TiO_2 for the huddle effect to occur.

However, the most commonly used artificial source of UV light in photocatalytic disinfection up to date is the conventional mercury low pressure (LP) and medium pressure (MP) UV lamps. These UV lamps are characterized with fixed wavelengths and other shortcomings like short bulb lifetime, low energy efficiency, and environmental pollution due to mercury [20]. Another alternative of the UV light source that can be applied in TiO_2 photocatalytic disinfection is the broadband UV−A lamp. However, this lamp consumes many times more electrical power and produces output wavelengths unnecessary to the task, i.e., a spectrum that is centered at 350 nm but extends from 300 to 400 nm [21]. The newly emerging ultraviolet light-emitting diodes (UV-LEDs) have shown the potential in replacing the conventional UV mercury lamps in water disinfection [22–24]. These UV-LEDs are characterized with the diversity in wavelengths ranging from near-ultraviolet light-emitting diodes, whose emission wavelength is approximately 300–400 nm, and deep-ultraviolet LEDs, whose emission wavelength is approximately 200–300 nm [25]. Other advantages of UV-LEDs include: environmental friendly (no mercury), compact and durable, faster start-up time, less energy consumption, longer lifetime and the ability to turn on and off with high frequency [25–27]. Unfortunately, in literature, there are only three reports that touched on the UV-LED/TiO_2 disinfection system for different bacteria.

Among the three reports, *Basillus subtilis* cells in suspension were disinfected using two UVA-LED at wavelengths of 370 and 377 nm in presence of TiO_2. It was found that, the longest UV wavelength that caused a marked decrease in the concentration of *B. subtilis* in a reasonable time was approximately 370 nm [28]. A novel UVA-LED/TiO_2 system was also successfully applied in the inactivation of antibiotic-resistant bacteria in both continuous and periodic illumination and compared with UVC disinfection (photolysis) and UVA mercury lamp/TiO_2. The wavelength of the UVA-LED and UVA mercury lamp was 365 nm. It was found that the UVA-LED/TiO_2 process was an attractive alternative considering the residual disinfection effect and energy consumption [29]. Recently, the disinfection of total coliforms that composed of *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella* in superficial water was performed using three sources of radiation, i.e., mercury vapor lamp, solar simulator and UVA-LED under photolysis and photocatalysis (using different catalysts: TiO_2, SiZnO, N-SiZnO, and F-N-SiZnO). The UVA-LED/TiO_2 was found to have the highest disinfection efficiency of 99% compared to 52% and 31% for the mercury vapor lamp and solar simulator, respectively [30]. The application of UVA-LED/TiO_2 in the above studies was regarded effective for microbial disinfection in water due to the high disinfection efficiency, residual disinfection effect, low energy consumption and the application of a single 385 nm (UVA) wavelength. Considering the unique feature of the diverse wavelengths of UV-LEDs, it is therefore necessary to carry out more studies in TiO_2 photocatalytic disinfection using different UV-LED wavelengths and compare the disinfection performance with their corresponding photolytic disinfection.

Besides, previous studies indicate that microorganisms, especially bacteria, suffer from UV light up to an instant inactivation [31]. However, bacterium especially *Escherichia coli* (*E. coli*) is known to repair UV-damaged DNA by mechanisms such as photoreactivation and excision repair/dark repair, which weakens the disinfection robustness for the long term operation [32,33]. Photoreactivation is a process where microorganisms utilize light in the wavelength range of 330–480 nm to activate, photolyase enzyme, in order to split CPD to recover the damaged DNA. Meanwhile, dark repair is a process which requires coordination of over a dozen proteins to excise and repair the damaged DNA segment [32,34]. In TiO_2 photocatalytic disinfection, the UV photons directly attack the bacteria coupled with the concomitant action of $OH^\bullet$ radicals photo-generated on the TiO_2 surface, rendering the repair mechanisms insufficient to protect the cells. Additionally, increasing irradiation time and/or light intensity in the application of UV/TiO_2 can cause “residual disinfecting effect” [13,15,28,35]. On the other hand, in UV photolytic disinfection, either high UV light intensity or MP mercury UV lamp has been suggested to inhibit the repair [36–38]. However, *Legionella pneumophila* was reported to photoreactivate nearly completely after irradiation with either LP or MP UV lamp [39]. Regarding the durability of either photolytic or TiO_2 photocatalytic disinfection, the repression of microorganisms’ repair after UV disinfection is a major concern.

In this work therefore, UV-LED photolytic and TiO_2 photocatalytic disinfection of the model microbe *E. coli* were investigated in a batch water disinfection system. Different parameters were considered such as UV-LED wavelengths, irradiance and TiO_2 concentrations. In addition, the durability of the disinfection was examined by investigating the repair of the *E. coli* through the photoreactivation and dark repair processes in both the UV-LED photolytic and TiO_2 photocatalytic disinfection. The results of this work should favor the deep understanding on the applicability of UV-LED photolytic and/or UV-LED/TiO_2 photocatalytic disinfection, and thereafter the appropriate design in various circumstances for water treatment.

2. Materials and methods

2.1. Bacteria culture

In this study, 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 hours until log phase was reached, determined by measurement of OD600 through the UV-1780 UV-VIS spectrophotometer (Shimadzu, Japan). 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 10^7 CFU/mL, determined by standard plate count method and OD600 measurement.

2.2. UV-LED and fluence determination

UV-LEDs with emissions at 265, 275, 310 and 365 nm and optical power output of 1.1, 2.2, 1.3 and 6.8 mW respectively at current of 20 mA achieved at voltages of 6.0, 5.3, 6.0, 3.9 V respectively (Great Bright Co. Ltd, China) were used. The emission spectra of the UV-LEDs were measured with Spectro 320 Optical Scanning Spectrometer and exhibited peak emission wavelengths at 265, 275, 309 and 364 nm. The UV-LEDs were soldered to a circular board of 50 mm diameter in a 24 mm square array consisting of three rows of three UV-LEDs in series. Irradiance was measured at the same water surface level using IL-1700 radiometer with SED 270 detector (International Light, USA). To achieve equivalent irradiance current was varied using 2400 Keithley Source meter and fluence was calculated from the product of irradiance and exposure time (in seconds) [40].

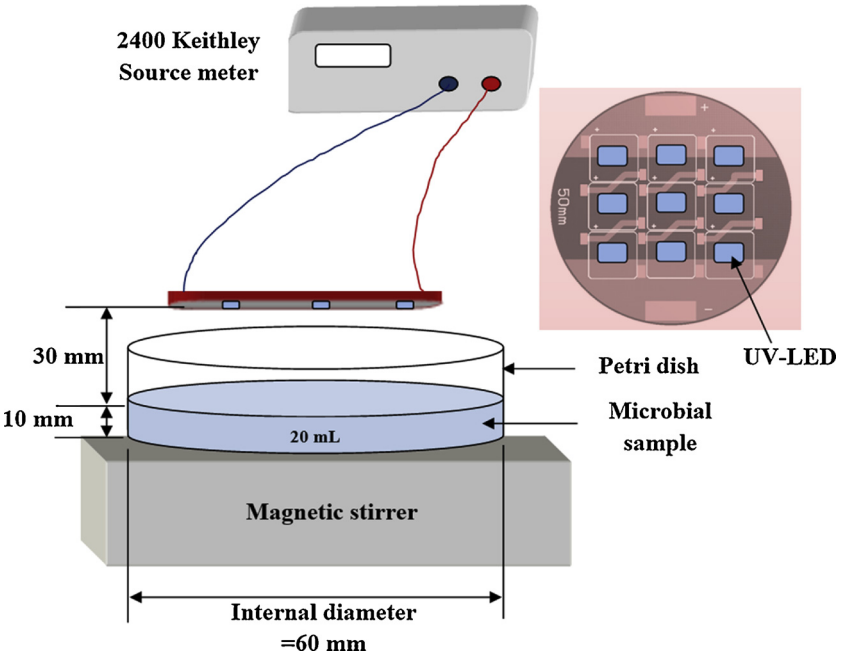

Fig. 1. Set-up of a batch photo reactor.

2.3. Photocatalytic reaction

The catalyst used in this study was TiO_2 anatase (Shanghai Macklin Biochemical Co., Ltd, China) with density 4.2 g/cm^3, a surface area of 50 m^2/g and average diameter size of 20–30 nm. To determine the optimal TiO_2 concentration to use in subsequent experiments, the catalyst was between 0.50 and 1.25 g/L, and 365 nm UV-LED was used as a radiation source at an irradiance of ~ 0.49 mW/cm^2. The stock aqueous TiO_2 suspension was always prepared by sonication immediately prior to photocatalytic experiment and kept in the dark. While being stirred with a sterile magnetic stir bar, 20 mL TiO_2-cell slurry was irradiated at 3 cm from the UV-LED source at designated irradiance and time intervals. Under the same conditions and volume, the same experiments were conducted with microbial suspension without TiO_2. While stirring for 40 min, the control experiments with or without TiO_2 were kept in the dark. All disinfection experiments were conducted at room temperature and were repeated three times for reproducibility of the results. Fig. 1 shows the set-up of a batch photo reactor used in this study.

2.4. Photoreactivation and dark repair process

Microbial samples were taken before and after UV irradiation for bacteria concentration determination and the rest UV irradiated samples were used for the photoreactivation and dark repair processes. While stirring with a sterile magnetic stir bar, the petri dishes containing the UV irradiated microbial samples were irradiated by fluorescent lamp (white light fluorescent lamp, 15 W; Philips) placed 30 cm above them for 8 h. The other samples were kept in the dark for dark repair under the same time and room temperature as those for photoreactivation. The samples were taken at 2-h intervals for bacteria concentration determination.

2.5. Cell viability assay

Prior to analysis, samples were not filtered to remove TiO_2 particles to avoid losses of bacteria during filtration. Three 100 µL samples were micropipetted from the samples that were serially diluted in 0.85% saline solution and plated onto three respective LB agar plates and spread over the agar surface using a plate spreader. Plates were then incubated for 24 h at 37 °C after which viable colonies observed were counted. An average count was calculated from the three plate counts.

2.6. Calculation

2.6.1. Inactivation quantification

Inactivation of *E. coli* was analyzed by calculating log inactivation using Eq. (4) and the values obtained plotted against irradiation time for analysis. When required, the fluence was determined as earlier explained in Section 2.2.

$$Log\ inactivation = Log\left(\frac{N_0}{N}\right) \tag{4}$$

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

In situations where N = 0, the percentage disinfection efficiency turns to be 100% as can be calculated from Eq. (5). Therefore, in this work, when N = 0, the inactivation was defined as “total” inactivation.

$$Disinfection\ efficiency\ (\%) = \frac{N_0 - N}{N_0} \times 100\% \tag{5}$$

2.6.2. Repair quantification

The percentage of repair (photoreactivation or dark repair) for the inactivated *E. coli* was quantified using Eq. (6) [37].

$$Percentage\ of\ repair\ (\%) = \frac{N_t - N}{N_0 - N} \times 100\,\% \tag{6}$$

in which N_t is the cell number (CFU/mL) after repair for a period of time, t. Note that, the degree of repair thus represents the fraction of the inactivated cells that has been recovered after disinfection. Therefore, the percentage of repair in Eq. (6) is developed to reflect the degree of the recovered cells (N_t-N) from either photoreactivation or dark repair among the total UV inactivated cells (N_0-N).

2.6.3. Electrical energy consumption quantification

To determine the amount of electrical energy consumed by the various UV-LEDs used in this work for the inactivation and repression of subsequent repair of *E. coli*, the electrical energy consumption ($E_{E,N}$) was calculated using Eq. (7) that considers fluence-response curves showing non-linear log reduction (e.g. shoulder, tailing or sigmoidal

curve) [41].

$$E_{E,N} = \frac{A \bullet F_N}{3.6 \bullet 10^3 \bullet V \bullet C \bullet WF} \quad (7)$$

in which $E_{E,N}$ is the electrical energy per specific N-log inactivation (kWh/m^3), A is the irradiated surface area (cm^2), F_N is the fluence required for N-log inactivation (mJ/cm^2). V is the sample volume (mL), C is the wall plug efficiency calculated using Eq. 8 (0.009, 0.021, 0.011, 0.087 for the 265, 275, 310 and the 365 nm UV-LEDs, respectively), WF is the water factor, accounting for the UV absorbance and depth of the water. The value 3.6×10^3 is to convert between hours and seconds, mW and kW, and mL and m^3.

$$C = \frac{P_{output}}{P_{input}} = \frac{P_{output}}{I_A \bullet V_A} \quad (8)$$

in which P_{output} is the UV-LEDs optical power (mW), P_{input} is the applied electrical power (mW), I_A is the applied current (mA) and V_A is the applied voltage (V).

3. Results and discussions

3.1. Evaluation on inactivation of E. coli with variable conditions in photolytic and photocatalytic disinfection

3.1.1. Effect of TiO_2 concentration

The comparative experiments without or with TiO_2 (i.e., photolytic and photocatalytic disinfection) showed that, after 40 min of stirring in the dark, no inactivation occurred. It indicats that the disinfection with or without TiO_2 in the dark does not occur (Fig. 2). So, any photolytic or photocatalytic disinfection observed in this study is exclusively attributed to the UV light derived from UV-LED.

A lag and exponential phase occurred for *E. coli* during the inactivation period (Fig. 2). For samples with TiO_2, the lag phase characterized by insignificant inactivation lasted for about 10 min. During this period, the UV photon absorbed by the DNA and the attack of the generated $OH^{\bullet}$ to the cell membrane could have led to insignificant damage due to the consistent auto-repair of the damaged DNA and cell membrane by the repair enzymes of *E. coli*. Immediately after the lag phase, the exponential phase started which was characterized by an accelerated inactivation (Fig. 2). The acceleration of inactivation can be attributed to more UV photon absorbed by DNA and the repeated $OH^{\bullet}$ attacks on the cell membrane of *E. coli*, resulting to severe damage of DNA and perforation of cell membrane respectively. Therefore, the repair enzymes were not able to auto-repair the damaged DNA or the cell membrane at this phase. It has also been reported in literature that, the acceleration of inactivation could also result from a photo-Fenton reaction phenomena, i.e., when the cell membrane is perforated, ferrous, and ferric ions could be released which react to form $OH^{\bullet}$ radical (Eqs. 9a-c) [14].

$$Fe^{+2} + H_2O_2 \rightarrow OH^{\bullet} + OH^{-1} + Fe^{+3} \quad (9a)$$

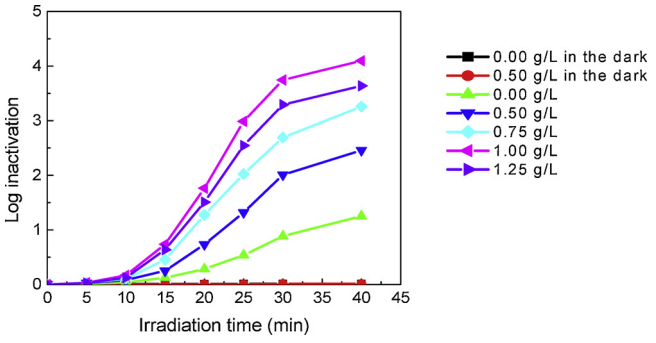

Fig. 2. Effect of TiO_2 concentration on inactivation of *E. coli* using 365 nm UV-LED, with curves in the dark as control.

$$Fe^{+3} + OH^{-1} \rightarrow Fe(OH)^{2+} \quad (9b)$$

$$Fe(OH)^{2+} \xrightarrow{h\nu} + Fe^{2+} + OH^{\bullet} \quad (9c)$$

For the samples without the TiO_2, the lag phase lasted about 15 min that was 5 min more (longer shoulder) than the lag phase time for the samples with TiO_2 (Fig. 2). It has been reported that, UVA can produce CPDs but in small amount [42,43] and in the recent study, it was reported that, UVA can sensitize the bacteria to overproduce RecA proteins leading to formation of more CPD [44]. Therefore, for significant amount of CPD to accumulate that can cause damage to the DNA, more time is needed which could have led to lag phase taking longer time in this case. In addition, other previous studies indicated that, UVA can induce $OH^{\bullet}$ which have lethal effects [45,46]. Therefore, the accumulation of the few CPD and $OH^{\bullet}$ generated by UVA could have led to the inactivation in the exponential phase observed (Fig. 2).

The maximum log inactivation of 4.1 was obtained for samples with 1.00 g/L of TiO_2. Meanwhile, the maximum log inactivation was 1.3, 2.5, 3.3, and 3.6 for samples with 0.00, 0.50, 0.75 and 1.25 g/L of TiO_2 respectively. As the concentration of TiO_2 increased from 0.00 to 0.100 g/L, the log inactivation increased over the irradiation time (Fig. 2). This indicates that, addition of TiO_2 in suspension leads to increase in inactivation rate of *E. coli* which can be attributed to the increase in the $OH^{\bullet}$ generated from the surface of the UV irradiated TiO_2. However, as the TiO_2 concentration increased from 1.00 to 1.25 g/L, there was a decrease in the inactivation rate indicating a detrimental effect of high concentration of TiO_2 on inactivation of the *E. coli* (Fig. 2). The detrimental effect can be attributed to either a screening effect of excess TiO_2 particles on the surface of *E. coli* [47] or the accumulation of $OH^{\bullet}$ radicals which at high local concentration, they readily dimerize to H_2O_2 (Eq. (2)) which in excess, can interact with $OH^{\bullet}$ to form hydroperoxyl radicals, $HO_2^{\bullet}$ (Eq. (10)) which does not contribute to the inactivation process due to their less reactivity [11]. Analogous results to this work were also reported in the previous studies [13–15,48].

$$H_2O_2 + OH^{\bullet} \rightarrow H_2O + HO_2^{\bullet} \quad (10)$$

3.1.2. Effect of irradiance

The irradiance of the 365 nm UV LEDs was varied between 0.49–1.98 mW/cm^2. For the samples without TiO_2, the inactivation was greatly accelerated as irradiance increased. The log inactivation of 1.3, 1.6, 2.0, 2.6 at irradiance of 0.49, 0.97, 1.48, 1.98 mW/cm^2 were obtained after 40 min, respectively (Fig. 3). The longer shoulder length was observed at low irradiance which can be attributed to insignificant damage of the bacteria hence some repair could have taken place. Higher log inactivation was obtained at higher irradiance within a shorter time which could have been be due to the high flow of photons at high irradiance which directly attack the *E. coli* bacteria and thus preventing its self-defense and auto-repair mechanisms from taking place leading to accelerated inactivation [13]. It was also reported that, at a high irradiance and shorter time, the repair enzymes are more affected than at low irradiance for longer time [49]. Therefore, it is appropriate to apply high irradiance for a shorter time as compared to low irradiance for a longer time. For the samples with TiO_2, the log inactivation after 40 min was 4.1, 4.3, 4.7, 5.0 at an irradiance of 0.49, 0.97, 1.48, 1.98 mW/cm^2, respectively. The inactivation rate increased and the shoulder length was shortened as irradiance increased from 0.49 to 1.48 mW/cm^2. This is because, a huddle effect is expected to take place even at low irradiance which will prevent the self-defense and auto-repair mechanisms of the *E. coli* bacteria from protecting the cells leading to increase in inactivation rate as irradiance increases. Although there was an observed increase in log inactivation as the irradiance increased from 1.48 to 1.98 mW/cm^2, the rate of increase was rather slow (Fig. 3). In fact, a slow rate of inactivation at higher irradiance was also reported in previous studies [13,28], which can be

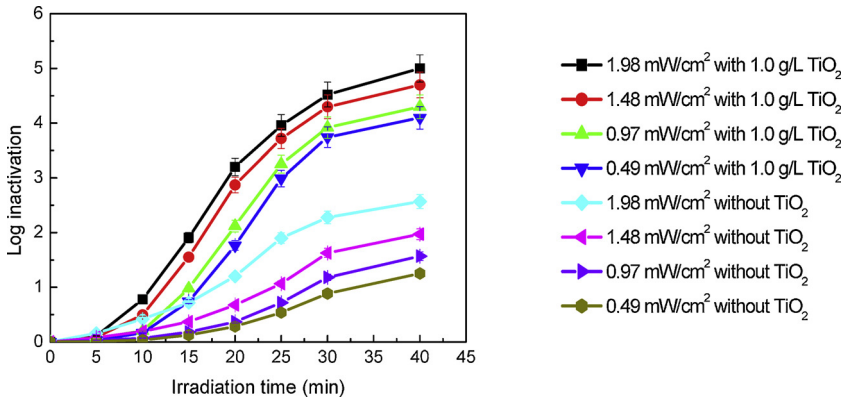

Fig. 3. Effect of irradiance on inactivation of *E. coli* using 365 nm UV-LED for photolytic and TiO_2 photocatalytic disinfection.

attributed to self-recombination of the excessive generated $OH^{\bullet}$ radicals which could have led to the decrease in the inactivation rate as earlier explained in literature [11].

3.1.3. Effect of wavelength

Disinfection efficiency in the absence and presence of TiO_2 under 265, 275, 310, 365 nm UV-LEDs was studied at similar irradiance of 0.49 mW/cm^2 and 1.00 g/L of TiO_2. The disinfection efficiency of *E. coli* was highest in both photolysis and TiO_2 photocatalysis when 265 nm UV-LEDs were used as a source of radiation, followed by 275, 310 and 365 nm UV-LEDs in succession (Fig. 4a and b). Similar results were also obtained in previous research work [14]. However, in another study, UVA/TiO_2 had higher inactivation efficiency than the LP mercury lamp UVC photolysis. That was because of the higher UV dose of 28 J/cm^2 for the UVA/TiO_2 as compared to lower UV dose of 8 mJ/cm^2 for the LP mercury lamp UVC photolysis [29]. Although the absorption spectrum of DNA for wavelengths > 300 nm is lower than that of wavelengths < 300 nm [50], the absorption spectrum of DNA at 310 nm is slightly higher than 365 nm and they can produce CPDs which could have led to the higher inactivation of the 310 nm than the 365 nm UV-LEDs [18]. The inactivation rate was increased in both the 310 and 365 nm UV-LEDs by the addition of TiO_2 (Fig. 4a). The increase is attributable to the huddle effect of the UV photons and $OH^{\bullet}$ which prevents any repair from protecting the cells hence, increasing inactivation rate. For the 265 and 275 nm UV-LEDs (Fig. 4b), the disinfection efficiency decreased with addition of TiO_2 which can be attributed to a screening effect by the TiO_2 which protected the *E. coli* bacteria against the strong UV photon of the UV-LEDs [47]. As previous studies showed, the UV light should provide energy equal to or greater than the TiO_2 band gap (e.g. B_g = 3.20 eV for Anatase TiO_2) for the $OH^{\bullet}$ radicals generation [8,9]. Otherwise, TiO_2 particles seem futile even worsen the penetration effect of the UV light. The finding of this study confirms the importance on the wavelength selection in TiO_2 photocatalytic systems.

"Total" inactivation was analyzed in both photolytic and TiO_2 photocatalytic disinfection. In photolysis, only 2.3 and 1.6 log inactivation was achieved by the 310 and 365 nm UV-LEDs respectively after 45 min of irradiation time. Therefore, longer time was required to achieve the "total" inactivation. However, after addition of the 1.0 g/L TiO_2, the log inactivation by the 365 nm UV-LEDs improved to 4.0 meanwhile, "total" inactivation was achieved by the 310 nm UV-LEDs after 40 min of irradiation time. After 45 min of irradiation, both the 365 and 310 nm UV-LEDs achieved "total" inactivation. On the other hand, "total" inactivation was achieved after 60 s and 75 s of irradiation time for the 265 and 275 nm UV-LEDs photolysis. And in TiO_2 photocatalysis, it required 75 and 90 s of irradiation time for the 265 and 275 nm UV-LEDs, respectively.

Summarily, the above results indicate that the 265 and 275 nm UV-LEDs require shorter time to achieve higher log or "total" inactivation as compared to the 310 and 365 nm UV-LEDs. It is worth noting that the addition of TiO_2 had a detrimental effect on the 265 and 275 nm UV-LEDS. However, for the 310 and 365 nm UV-LEDS, the addition of TiO_2, improved the disinfection efficiency significantly.

3.2. Evaluation on repair of E. coli in photolytic and photocatalytic disinfection

After photolytic and TiO_2 photocatalytic disinfection, the repair due to photoreactivation and dark repair of the *E. coli* bacteria was analyzed after similar 1.0, 4.0 log and "total" inactivation was achieved. At "total" inactivation, no repair occurred in both photolysis and TiO_2 photocatalysis, which was similarly observed in previous study [14]. Percentage of photoreactivation was first analyzed after 1.0 log inactivation was achieved. In photolysis, the percentage of photoreactivation of 17.5%, 15.4%, 12.5% and 11% was attained after 8 h of photoreactivation time when the 310, 265, 275 and 365 nm UV-LED irraiation was applied, respectively (Fig. 5a). On the other hand, the

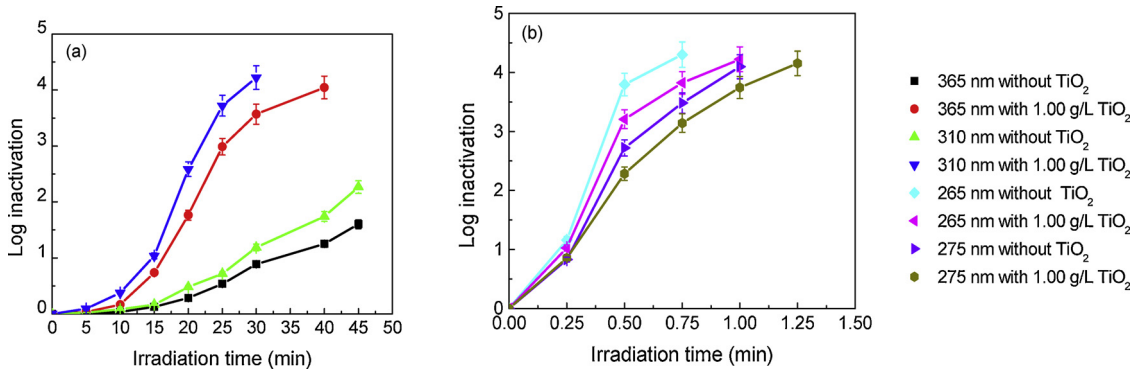

Fig. 4. Log inactivation of *E. coli* using (a) 310, 365 nm and (b) 265, 275 nm UV-LEDs.

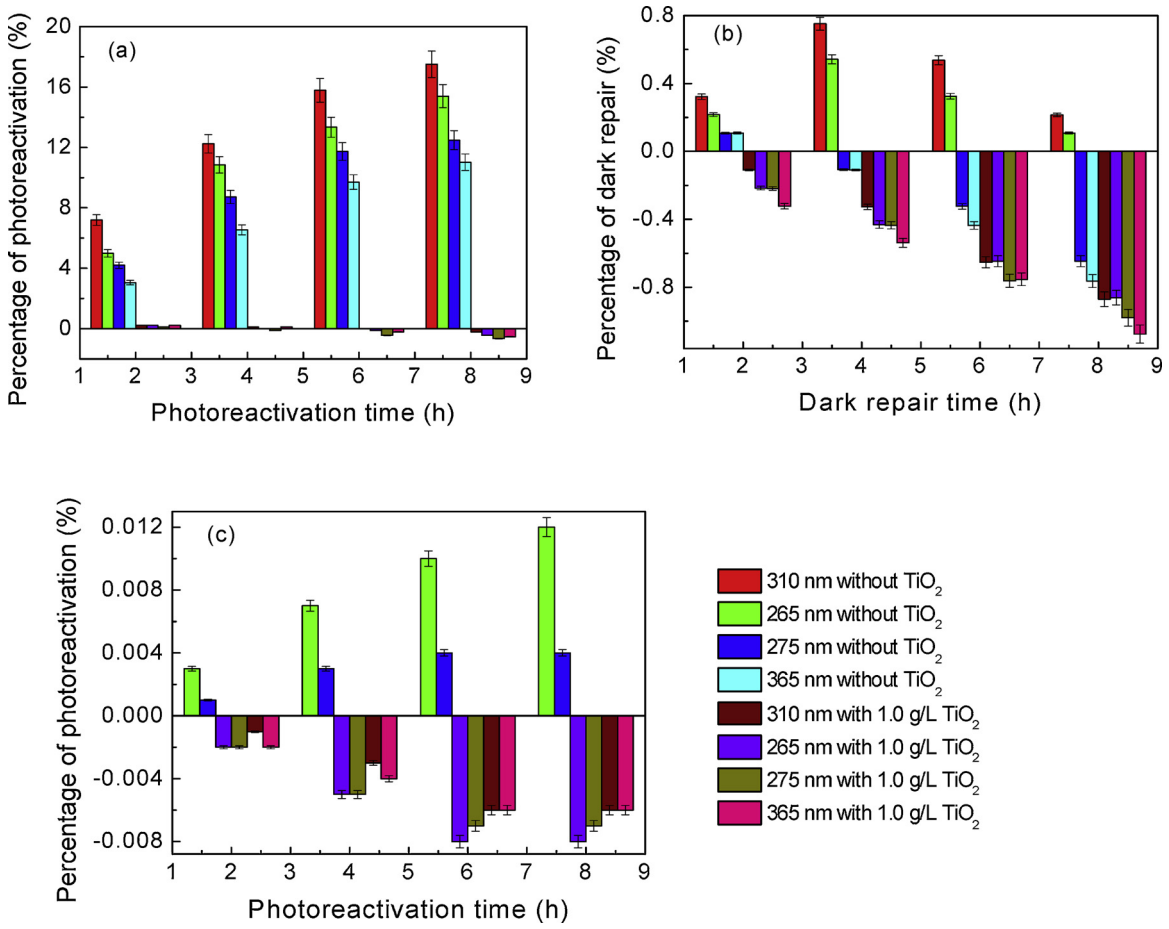

Fig. 5. Repair of *E. coli* after photolytic and TiO_2 photocatalytic disinfection. (a) photoreactivation after 1.0 log inactivation, (b) dark repair after 1.0 log inactivation and (c) photoreactivation after 4.0 log inactivation.

percentage of dark repair was 0.32%, 022%, 0.11% and 0.11% after 2 h of dark repair time when the 310, 265, 275 and 365 nm UV-LED irradiation was applied, respectively. A maximum percentage of dark repair of 0.75% and 0.54% was only attained in the respective 310 and 265 nm UV-LED irradiation after 4 h dark repair time. After the 4 h, a decay phase (mortality) occurred in all the UV-LED irradiations (Fig. 5b). The mortality observed can be attributed to a residual effect of radiation on the bacterial DNA, since the biochemical mechanism of actuation needs some time to be manifested completely. Note that, mortality was not observed after the 8 h in the photoreactivation experiments. This observation can be attributed to the repair of damaged DNA being more effective within the photoreactivation time [38]. Compared to photoreactivation, the dark repair occurred to a lesser extent which demonstrates that, photo-effect is the dominant mechanism of repair, an observation that was similarly observed in the previous studies [51–53]. The repair observed at this low 1.0 log inactivation in photolysis, it could be due to insufficient damage of the *E. coli* resulting to some repair taking place. In the case of TiO_2 photocatalytic disinfection, insignificant percentage of photoreactivation about 0.2% after 2 h of photoreactivation followed by a decay phase (mortality) occurred after the 1.0 log inactivation (Fig. 5a). The insignificant percentage of photoreactivation in the presence of TiO_2 was similarly reported in previous studies [14,15,29]. The observation can be attributed to the concomitant effect of the UV photon and the $OH^{\bullet}$ generated from the surface of UV irradiated TiO_2 that could have led to more damage to the *E. coli* than the UV photons alone in photolysis. In addition, the observed mortality can be attributed to the residual disinfecting effect of the $OH^{\bullet}$ [29]. For the UV-LEDs in both photolytic and photocatalytic disinfection, a higher percentage of repair was attained after the 310 and 265 nm irradiation and the least was obtained after the 275 and 365 nm irradiation. The lower percentage of repair at the 275 and 365 nm irradiation can be attributed to detrimental effects on the cell like i) the protein damage by the 275 nm since proteins show absorption maximum between 275–280 nm [54]; and ii) the oxidative disturbance of bacterial membranes induced by the 365 nm [55,56].

At 4.0 log inactivation, photolysis was only considered for the 265 and 275 nm UV-LEDs because, the 310 and 365 nm UV LEDs required relatively longer time to achieve the same log inactivation. Therefore, in the photolytic disinfection, only photoreactivation occurred while dark repair did not (Fig. 5c). The maximum percentage of photoreactivation in the 265 nm UV-LED irradiation was 0.012% which was higher than 0.004% in the 275 nm UV-LED irradiation after 8 h of photoreactivation. At this 4.0 log inactivation, the DNA could have been sufficiently damaged by the UV photon in photolysis leading to repression of dark repair. However, the damage was not enough to repress completely the photoreactivation which explains the absence and low percentage of photoreactivation observed (Fig. 5c). Meanwhile, neither photoreactivation nor dark repair occurred in the case of TiO_2 photocatalytic disinfection and only a decrease in viable colonies of *E. coli* bacteria was observed characterized by negative percentage of repair due to mortality (Fig. 5c). The repressed photoreactivation and dark repair in TiO_2 photocatalysis could be attributed to the severe damage of the DNA by the UV photon due to high UV dose at 4.0 log inactivation [37,38] and severe damage of the cell membrane and oxidative attack of intracellular components by the $OH^{\bullet}$ generated from the surface of the UV irradiated TiO_2 that could have led to a subsequent cell death [4].

Notably, no dark repair was observed in all the TiO_2 photocatalytic

Table 1
Time, UV dose and electrical energy required to achieve similar 1.0 log, 4.0 log and "total" inactivation in direct photolytic and TiO_2 photocatalytic disinfection at optimal UV irradiance of 0.49 mW/cm^2.

UV-LED wavelength (nm)	TiO_2 (g/L)	Time (s)			UV dose (mJ/cm^2)			$E_{E,N}$ (kWh/m^3)		
		1.0 log inactivation	4.0 log inactivation	"Total" inactivation	1.0 log inactivation	4.0 log inactivation	"Total" inactivation	1.0 log inactivation	4.0 log inactivation	"Total" inactivation
265	0	12	45	60	5.88	22.05	29.40	0.26±0.017	0.97±0.063	1.30±0.083
	1.0	15	60	75	7.35	29.40	36.75	0.32±0.021	1.30±0.083	1.62±0.108
275	0	15	60	75	7.35	29.40	36.75	0.14±0.004	0.55±0.017	0.69±0.021
	1.0	18	75	90	8.82	36.75	44.10	0.17±0.005	0.69±0.021	0.83±0.025
310	0	1680	na	na	823	na	na	29.38±1.573	na	na
	1.0	900	1500	2400	441	735	1176	15.74±0.851	26.24±1.406	41.98±2.239
365	0	1800	na	na	882	na	na	3.98±0.045	na	na
	1.0	900	2400	2700	441	1176	1323	1.99±0.024	5.31±0.060	5.97±0.67

[na]Longer time was required to achieve similar 4.0-log and "total" inactivation, hence both the UV dose and electrical energy consumption values were not calculated.

experiments (Fig. 5). This could be explained by two main reasons, i) the photo-sensitivity of the *E. coli*, since photo-effect has been known to be the dominant mechanism of repair [51–53]; and ii) the residual disinfection effect of the $OH^{\bullet}$ generated from the surface of the UV irradiated TiO_2 [29].

3.3. Evaluation on electrical energy consumption in photolytic and photocatalytic disinfection

At similar N-log inactivation, the 275 nm UV-LED required lower electrical energy consumption ($E_{E,N}$) as compared to the other UV-LEDs in both photolytic and photocatalytic disinfection (Table 1). This can be attributable to its germicidal efficiency and higher wall plug efficiency of 0.021. Although the 265 nm UV-LED had a higher germicidal efficiency than all the other wavelengths (Fig. 4b), its lower wall plug efficiency of 0.010 led to higher $E_{E,N}$ as compared to the 275 nm UV-LED which was similarly reported in Refs. [41,52,57]. The 365 nm UV-LED required a lower $E_{E,N}$ compared to the 310 nm UV-LED. This can be attributed to the higher wall plug efficiency of 0.087 and the lower $E \approx 5.45 \times 10 - 19$ J required to cause the TiO_2 (Anatase, $B_g = 3.20$ eV) oxidation by the 365 nm UV-LED compared to the 310 nm UV-LED which has a wall plug efficiency of 0.011, and will require $E \approx 6.42 \times 10 - 19$ J to cause the TiO_2 oxidation. The higher $E_{E,N}$ of the 310 and 365 nm UV-LEDs compared to the 265 and 275 nm UV-LEDs, can be ascribed more to their lower germicidal effect, hence, their disinfection was mainly accelerated by TiO_2 (Fig. 4a).

4. Conclusions

Direct photolytic and TiO_2 photocatalytic disinfection with respect to the inactivation and the repression on the subsequent repair of *E. coli* were compared at different TiO_2 concentration, irradiance and UV-LED wavelengths. Inactivation in photolytic disinfection was more effective when the 265 and 275 nm UV-LEDs were used. Although the addition of TiO_2 in suspension had a detrimental effect in the inactivation at the wavelengths of 265 and 275 nm, it improved significantly the inactivation efficiency of the 310 and 365 nm UV-LEDs. Notably, there was only an insignificant photoreactivation and no dark repair after 1.0 log inactivation and no repair (photoreactivation and dark repair) occurred at all after the 4.0 log and "total" inactivation in all the tested TiO_2 photocatalytic disinfection. Meanwhile, the repair was only repressed after achieving "total" inactivation in the case of photolytic disinfection. Comparing the wavelength, the 275 and 365 nm UV-LEDs had a lower percentage of repair in both photolytic and photocatalytic disinfection. In addition, the 275 nm UV-LEDs required a lower electrical energy consumption in both photolytic and photocatalytic disinfection. To avoid any suspected repair of pathogens after UV irradiation as the one observed at 1.0 and 4.0 log inactivation in the case of photolytic disinfection, the TiO_2 can be provided in immobilized form in the future UV-LED disinfection devices. Apart from its repressive effect, the immobilized TiO_2 will help in reducing the cost of dispersing and removing TiO_2 from the treated water. Therefore, the provision of TiO_2 in immobilized form and using the 275 nm UV-LED as a radiation source appear a viable option to be applied for water disinfection.

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