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The role and synergistic effect of the light irradiation and H_2O_2 in photocatalytic inactivation of *Escherichia coli*

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ABSTRACT

Inactivation of *Escherichia coli* K-12 was conducted by applying a continuous supplying of commercial H_2O_2 to mimic the H_2O_2 production in a photocatalytic system, and the contribution of H_2O_2 in photocatalytic inactivation was investigated using a modified "partition system" and five *E. coli* mutants. The concentration of exogenous H_2O_2 required for complete inactivation of bacterial cells was much higher than that produced *in-situ* in common photocatalytic system, indicating that H_2O_2 alone plays a minor role in photocatalytic inactivation. However, the concentration of exogenously produced H_2O_2 required for effective inactivation of *E. coli* K-12 was much lower when the light irradiation was applied. To further investigate the possible physiological changes, inactivation of *E. coli* BW25113 (the parental strain), and its corresponding isogenic single-gene deletion mutants with light pretreatment was compared. The results indicate that light irradiation increases the bacterial intracellular Fe^{2+} level and favors hydroxyl radical (·OH) production via the catalytic reaction of Fe^{2+}, leading to increase in DNA damage. Moreover, the results indicate that the properties of light source, such as intensity and major emission wavelength, may alter the physiology of bacterial cells and affect the susceptibility to *in-situ* resultant H_2O_2 in the photocatalytic inactivation processes, leading to significant influence on the photocatalytic inactivation efficiencies of *E. coli* K-12.

1. Introduction

Photocatalysis has received increasing research interest in recent years as it can be conducted under ambient conditions (i.e. room temperature and one atmospheric pressure) and using sunlight as the light source, which allows the inactivation of microorganisms in water at low-cost [1]. Furthermore, photocatalysis produces less disinfection byproducts than those of the commonly used disinfection process such as chlorination [2].

To apply photocatalysis in water treatment and disinfection, a detail inactivation mechanism of microorganisms has to be investigated. Various reactive oxidative species (ROSs) have already been reported to effectively inactivate microorganisms in different photocatalytic systems [3–6]. In general, ·OH is believed to be the major ROSs responsible for the inactivation of bacteria [7–9]. However, recent studies have shown that other ROSs such as h^+ and H_2O_2 or even photogenerated e^- can also play important roles in the photocatalytic bacterial inactivation, depending on the light source and photocatalysts involved [3–8]. Although the role of *in-situ* resultant H_2O_2 in photocatalytic bacterial inactivation is still not clearly or completely elucidated, it is generally believed that H_2O_2 inactivates the bacterial cells by forming of strongly oxidant chemical species (i.e. ·OH) inside the bacterial cytoplasm instead of direct oxidizing the bacterial cells [10–12]. Wang et al. [9] investigated the roles of different ROSs by the addition of different scavenger for specific ROS in a partition system using B–Ni-codoped TiO_2 microsphere as photocatalyst and the results showed that H_2O_2 is the most important ROSs responsible for the bacterial inactivation even though there was only a low concentration of H_2O_2 (~5 μM) detected in the system. Another study using natural sphalerite for photocatalytic bacterial inactivation also reported *in-situ* resultant H_2O_2 is also important in the bacterial inactivation process while only a low concentration of *in-situ* resultant H_2O_2 is detected (<10 μM) [3]. According to Labas et al. [12], H_2O_2 concentration less than 1.176 mM (40 ppm) is not effective to the inactivation of *Escherichia coli*. Thus, to explain the effective inactivation in photocatalytic inactivation with *in-situ* resultant low H_2O_2

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concentration, Wang et al. [9] speculated that the continuous H_2O_2 supply in the photocatalytic system is the major reason and believed that the bacterial cells would be inactivated by *in-situ* resultant H_2O_2 instantly, with a low concentration detected in the system. However, there is no study on the kinetic of bacterial inactivation by *in-situ* resultant H_2O_2 in a continuous mode to support this hypothesis.

The light irradiation to photocatalytic inactivation was also found to be an important parameter to the photocatalytic inactivation. Chen et al. [13] reported that the disinfection efficiency of natural sphalerite is greatly influenced by light spectrum, light intensity as well as major emission wavelength of the light source. Wang and Lim [14] also reported that color of LED lamps greatly influence the photocatalytic bacterial inactivation efficiency of AgBr/Ag/TiO_2. However, these studies only had limited information on the effect of light irradiation on the ROS production, and the influence of light irradiation on the change of physiology of bacterial cells was never attempted.

To better understanding on the of photocatalytic inactivation mechanism of bacteria, the present study aims to investigate the roles of H_2O_2 and light source in the photocatalytic inactivation in a modified partition system with light pretreatment and a genetic study. The inactivation efficiency of *E. coli* with continuously supplying of H_2O_2 in the presence and absence of light irradiation was compared. The effect of light intensity and major emission wavelength of light source on H_2O_2 inactivation were also investigated in detail. Finally the possible inactivation mechanisms of *E. coli* was proposed by compared the inactivation of *E. coli* BW25113 (the parental strain) alone with four corresponding isogenic single-gene deletion mutants with light pretreatment.

2. Experimental section

2.1. Materials

Regenerate cellulose (RC) membrane (Molecular weight cut off: 12k Daltons) were purchased from Spectrum®Laboratories, Inc (USA). $FeSO_4$ and coumarin were purchased from Sigma–Aldrich Company (USA).

E. coli K-12 was obtained from the American Type Culture Collection. *E. coli* BW25113 (the parental strain) and its isogenic deletion mutants, *E. coli* JW0576-2, *E. coli* JW-588-1. *E. coli* JW0797-1 and *E. coli* JW1721-1, were purchased from Coli Genetic Stock Center (CGSC, Yale University, USA) [15]. The information of *E. coli* BW25113 and the deletion mutants is listed in Table 1. All the *E. coli* in the study (including, K-12, parental strain BW25113 and mutants) were cultured in Nutrient Broth (yeast extract: 3 g, peptone: 5 g, M-Lab, U.K.) in 37 °C for 12 h. The concentration of *E. coli* was around 2×10^9 colony forming unit (CFU) mL^{-1}.

2.2. Photocatalytic inactivation with continuous H_2O_2 supply in the partition system

The partition system used in this study was designed by modifying the setup reported in previous studies [16,17]. The partition system consists of two compartments: the photocatalyst compartment and bacterial cell compartment. Solution of different concentration of H_2O_2 was added into the photocatalyst compartment, while bacterial cells suspended in sterile saline solution were added into the bacterial cell compartment. The two compartments were separated by a piece of semi-permeable membrane that only allows small molecule, such as H_2O_2, to pass through. The volume of each compartment was 40 mL (Fig. 1). The partition system was put on a shaking panel running at 180 rpm to ensure proper mix and suspension of the bacterial cells and photocatalyst throughout the bacterial inactivation processes. H_2O_2 of constant concentration in a reservoir was continuously added into the photocatalyst compartment and the same volume of solution in the photocatalyst compartment was continuously pumped out by a peristaltic pump so as to maintain the constant concentration of H_2O_2 and volume of solution in the photocatalyst compartment (Fig. S1 in Supplementary Information).

The inactivation of *E. coli* K-12 was conducted by using a 200 W Xenon lamp (Beijing Perfect Light Co. Ltd., Beijing) with a UV cutoff filter ($\lambda < 400$ nm) as the light source. The visible-light (VL) intensity was measured by a light meter (LI-COR, USA) and adjusted at 200 mW cm^{-2}. The bacterial cells and photocatalyst were suspended in sterilized 0.9% NaCl (saline) solution and the initial cell density was adjusted to 2×10^5 CFU mL^{-1} by making a 10,000 fold dilution of the cultured *E. coli* K-12. One mL sample solutions were sampled from the bacterial cell compartment in different time

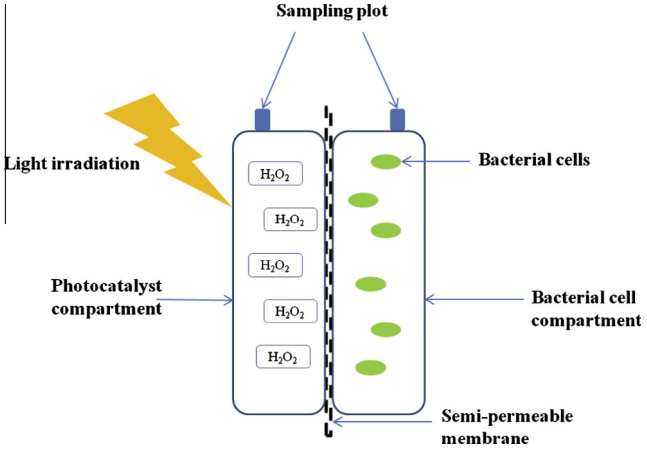

Fig. 1. Schematic illustration of the photocatalytic inactivation reactor.

Table 1
The genetic information of *Escherichia coli* parental strain (*E. coli* BW 25113) and its isogenic single-gene deleted mutants used in the present study.

Strain name	GCSC number	Deleted gene	GCSC mutation name	Mutation function
E. coli BW25113	7636	None	Not applicable	Not applicable
E. coli JW0576-2	8695	fes	fes722(del)::kan	Expression of ferric enterochelin esterase (Fes) Fes catalyzes the hydrolysis of the enterochelin moiety of ferric-enterochelin to yield ultimately three molecules of *N*-2,3-dihydroxybenzoylserine
E. coli JW0588-1	11768	entA	entA734(del)::kan	Expression of enterobactin A (EntA) EntA is a component of 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase which involve in the catalyzing of the synthesis of 2,3-dihydroxybenzoic acid from chorismic acid
E. coli JW0797-1	8844	dps	dps784(del)::kan	Expression of stress response DNA-binding protein (Dps) Dps binds to DNA non-specifically and protect DNA against oxidative stress
E. coli JW1721-1	9453	katE	katE731(del)::kan	Expression of catalase hydroperoxidase II (KatE) KatE employs a two-electron transfer in the dismutation of H_2O_2 into O_2 and H_2O

intervals and spread on nutrient agar plate (Lab M Ltd., UK) after appropriate serial dilution. Then the plates were incubated at 37 °C for 24 h for the analysis of cell survival (Detection limit: 1 CFU mL^{-1}). Light controls (light alone without H_2O_2) and dark (H_2O_2 alone without light) controls were also carried out for each set of experiment. All experimental controls and treatments were performed in triplicates.

The effect of H_2O_2 concentration, with or without the light irradiation, was compared and the effect of light intensity and major emission wavelength of the VL were also compared. Light intensity was controlled by adjusting the output power of the Xenon lamp. Light emitting diodes (LED) lamps with different emission wavelength were used to study the effect of the major emission wavelength of the light source fixed at 6 mW cm^{-2}. The light spectrum of the light source is showed in Fig. S2 (in Supplementary Information).

2.3. Effect of light pretreatment duration on E. coli K-12

E. coli K-12 (2×10^5 CFU mL^{-1}, prepared by making a 10,000 fold dilution of the bacterial culture) suspended in 50 mL saline solution were exposed to the light for 1 h in a 100-mL beaker, then the irradiation was stopped, and the H_2O_2 was added to a final concentration of 2 mM. The inactivation efficiency of these bacteria was compared with that of the dark controls (with no light pretreatment and stood in dark for 2 h before the addition of H_2O_2). All the experiments were conducted in triplicates.

2.4. Effect of light pretreatment on the response to different E. coli mutants

To investigate the possible physiological changes of the bacterial under the light irradiation, the inactivation efficiencies between *E. coli* BW25113 (the parental strain) and its isogenic deletion mutants, *E. coli* JW0576-2, *E. coli* JW-588-1. *E. coli* JW0797-1 and *E. coli* JW1721-1, with light pretreatment were compared. The mutants are related to intracellular iron regulation, DNA protection and H_2O_2 removal which are possibly related to the enhancement of inactivation under light irradiation. The parental strain and the deletion mutants are all derived from *E. coli* K-12 and each of the mutants is isogenic to the parental strain except the specific gene deletion [15]. Therefore, by comparing the bacterial inactivation by H_2O_2 of different mutants and the parental strain, the function of the specific gene in the light pretreatment can be revealed.

Different *E. coli* mutants (2×10^5 CFU mL^{-1}, by making a 10,000 fold dilution of the bacterial culture of respective mutants) were suspended in 50 mL saline solution were first exposed to the light for 1 h in a 100-mL beaker, then the irradiation was stopped, and the H_2O_2 was added to a final concentration of 2 mM. The inactivation efficiency of these bacteria was compared with that of the dark controls (with no light pretreatment). All the bacterial inactivation experiments were conducted in triplicates.

2.5. ROS determination

H_2O_2 was determined by modifying the method developed by Abbas et al. [18]. 0.4 mL samples/standards, 0.1 mL $FeSO_4$ and 0.1 mL coumarin were added into 0.4 mL citrate buffer (0.1 M, pH 3) and stood for 10 min in dark, and the final concentration of $FeSO_4$ and coumarin was 0.25 and 0.5 mM, respectively. The product (7-hydroxylcoumarin) was measured at an emission wavelength 456 nm with an excitation wavelength at 346 nm. The H_2O_2 concentration of the samples was obtained from the standard curve generated from the standards (Detection limit: 1 μM).

The generation of ·OH was determined by terephthalic acid. The ·OH was captured by terephthalic acid to produce a fluorescent product 2-hydroxylterephthalic acid [9]. The product was measured by an Infinite M200 fluorescence spectrophotometer (Tecan, Switzerland) at emission wavelength 425 nm with excitation wavelength at 315 nm.

The generation of $\cdot O_2^-$ was determined by the nitroblue tetrazolium (NBT) assay [19]. Formation of $\cdot O_2^-$ converts the soluble colorless NBT into insoluble purple formazan. The decrease of NBT was determined by a UV–Vis spectrophotometer at absorbance 259 nm.

2.6. Inactivation kinetic

To compare the rate of inactivation of *E. coli*, the inactivation kinetic was calculated according the model proposed by Geeraerd et al. [20]. The inactivation kinetic of the parental strains and mutants are fitted into the shoulder-linear-tail (Eq. (1)) with the software GInaFit [21].

$$\log(N) = \log\left[\left(10^{\mathrm{Log(N0)}} - 10^{\mathrm{Log(N_{res})}}\right) \times e^{-K_{max}t} \times \frac{e^{(K_{max}S_l)}}{1 + (e^{K_{max}S_l} - 1) \times e^{(-K_{max}t)}} + 10^{\mathrm{Log(N_{res})}}\right] \quad (1)$$

where K_{max} = specific inactivation rate (h^{-1}). S_l = shoulder length (h). N_{res} = residue population density (CFU mL^{-1}).

2.7. Statistical analysis

The differences of bacterial inactivation in treatment were statistically analysis with one-way Analysis of Variance (ANOVA) with post-hoc Tukey-b test. *P*-value with lower than 0.05 ($p < 0.05$) are regarded as significantly different from each other.

3. Results and discussion

3.1. Inactivation of E. coli using continuous H_2O_2 supply

The inactivation efficiency with different H_2O_2 concentration in photocatalyst compartment without light irradiation is showed in Fig. 2a. *E. coli* K-12 was found to be completely inactivated within 8 h. However, the required concentration of H_2O_2 in photocatalyst compartment was 8 mM, which is much higher than that detected in the common photocatalytic system. The inactivation efficiency decreased when the applied H_2O_2 concentration decreased, and no inactivation was observed when H_2O_2 concentration was lower than 2 mM. The rate of inactivation (K_{max}) increases and shoulder length (S_l) decreases when the applied H_2O_2 concentration increases (Table 2a) and the difference of the bacterial cells density at 8 h are statistically significant (one-way ANOVA, $p < 0.05$). Fig. 2b shows that the diffusion of H_2O_2 in the system, which gradually increased within the first 4 h and then leveled off afterward. The diffusion rate is similar to H_2O_2 production rate in photocatalytic system reported in our previous studies [9,22].

However, when light irradiation was applied to the system, the required H_2O_2 concentration for bacterial inactivation decreased significantly. Fig. 3a and b show the inactivation efficiency and concentration of H_2O_2 with different H_2O_2 concentration in photocatalyst compartment in the presence of light irradiation. Four-log inactivation was also observed within 8 h when 400 μM H_2O_2 was applied in the photocatalyst compartment when bacterial cells were under light irradiation. Similar to the inactivation in dark condition, K_{max} increases and S_l decreases when the applied H_2O_2 concentration increases under light irradiation (Table 2b) and the

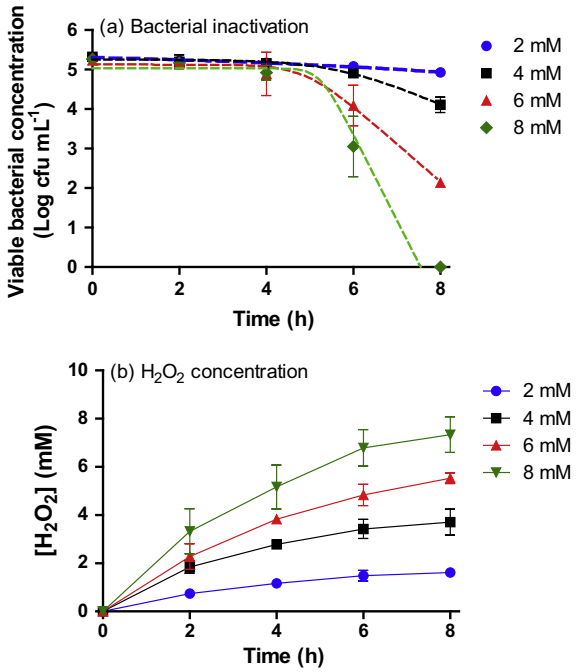

Fig. 2. (a) Inactivation of *Escherichia coli* K-12 and (b) H_2O_2 concentration in bacterial cell compartment with continuous H_2O_2 supply in dark. Initial *E. coli* K-12 concentration = 2×10^5 CFU mL^{-1}; Detection limit of *E. coli* K-12 = 1 cfu mL^{-1}. The point in (a) are fitted into the "shoulder-log" model proposed by Geeraerd et al. [20,21]. The error bars represent the standard deviation of replicates ($n = 3$).

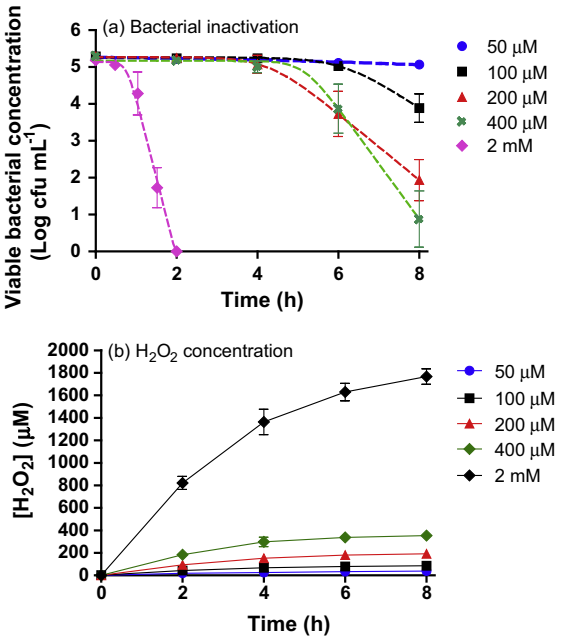

Fig. 3. (a) Inactivation of *Escherichia coli* K-12 and (b) H_2O_2 concentration in bacterial cell compartment with continuous H_2O_2 supply under light irradiation. Initial *E. coli* K-12 concentration = 2×10^5 CFU mL^{-1}; Light source, a Xenon Lamp with UV-filter ($\lambda < 400$ nm) with intensity of 200 mW cm^{-2}; Detection limit of *E. coli* K-12 = 1 CFU mL^{-1}. The point in (a) are fitted into the "shoulder-log" model proposed by Geeraerd et al. [20,21]. The error bars represent the standard deviation of replicates ($n = 3$).

differences between the bacterial cells density are statistically significant (one-way ANOVA, $p < 0.05$).

The bacterial inactivation by H_2O_2 fits well into a "shoulder-log" kinetic model. A lag phase (shoulder length) was always appeared. It is because H_2O_2 inactivates the bacterial cells by forming of strongly oxidant chemical species (i.e. ·OH) inside the bacterial cytoplasm instead of direct oxidizing the bacterial cells [10–12]. Therefore, a lag time is required for the H_2O_2 to diffuse across the cell membrane and accumulate in the cytoplasm. Labas et al. [12] studied the kinetic of bacterial inactivation by H_2O_2 in batch mode and reported that concentration of H_2O_2 lower than 1.176 mM is not effective for bacterial inactivation. The results of this study agree with their results, i.e. H_2O_2 of concentration lower than 2 mM is not effective for bacterial inactivation. Although the H_2O_2 was supplied in continuous mode in this study, no enhancement was observed in bacterial inactivation efficiency as comparing with the results of Labas et al. [12].

When light irradiation was applied to the system, the bacterial inactivation efficiency was greatly increased. Comparing the bacterial inactivation by 2 mM H_2O_2, no significant inactivation was observed in dark, while a much faster inactivation was achieved under light irradiation (K_{max}: 9.91 h^{-1}). Notably, the K_{max} of bacterial inactivation by 400 µM H_2O_2 (3.46 h^{-1}) under light irradiation was comparable to that of 8 mM H_2O_2 (3.45 h^{-1}) in dark (thought the S_l is slightly longer). The results showed that light irradiation is a more important factor in the enhancement of bacterial inactivation by H_2O_2, but not the mode of H_2O_2 supply.

Synergism of H_2O_2 and light irradiation has been extensively reported [23–26]. UV irradiation or sunlight can photolyse the H_2O_2 into more powerful ·OH. However, the light source used in this experiment is a Xenon lamp with UV-filter ($\lambda < 400$ nm), the proton should not be powerful enough to convert H_2O_2 into ·OH. Moreover, we had used different chemical probes to detect the generation of ROS, but ·OH or $\cdot O_2^-$ were not detected during the process. Therefore, the results indicate that light irradiation can facilitate the bacterial inactivation through H_2O_2 by other mechanisms (i.e. change of physiology of bacteria), which will be discussed in latter section.

There are various studies showing H_2O_2 is the major ROS responsible for the photocatalytic bacterial inactivation [3,27]. However, the concentration of H_2O_2 detection in their system was very low (<10 µM). According to the results of this study, no significant inactivation was observed when 50 µM of H_2O_2 was applied even in presence of strong light irradiation (200 mW cm^{-2}). Therefore, the low H_2O_2 level *in-situ* produced in most photocatalytic inactivation systems (usually <60 µM) [3,9,22] should not lead to efficient bacterial inactivation. The

Table 2
Variables and adjusted R^2 of the kinetic model of bacterial inactivation by H_2O_2 under different applied H_2O_2 concentration in (a) dark and (b) under light irradiation[a].

(a) In dark			
$[H_2O_2]$ (mM)	K_{max} (h^{-1})	S_l (h)	Adjusted R^2
2	0.25 ± 0.09	6.22 ± 1.27	0.9630
4	1.14 ± 0.17	5.76 ± 0.34	0.9797
6	2.21 ± 0.26	4.91 ± 0.32	0.9855
8	3.45 ± 0.24	4.58 ± 0.20	0.9955
(b) Under light irradiation[b]			
$[H_2O_2]$ (µM)	K_{max} (h^{-1})	S_l (h)	Adjusted R^2
50	0.23 ± 0.16	9.57 ± 0.94	0.8884
100	1.72 ± 0.09	6.19 ± 0.10	0.9979
200	2.07 ± 0.03	4.30 ± 0.05	0.9998
400	3.46 ± 0.24	5.15 ± 0.16	0.9941
2000	9.91 ± 0.88	0.77 + 0.09	0.9875

± denote the standard error of the variables.
[a] The kinetic of bacterial inactivation by H_2O_2 are fitted into a "should-log" model proposed by Geeraerd et al. [20,21].
[b] The Light source is a Xenon lamp with UV-filter ($\lambda < 400$ nm).

reason for the major role of H_2O_2 in some photocatalytic inactivation system requires further investigation.

3.2. Effect of light intensity and major emission wavelength

To further investigate the effect of the property of light source, the bacterial inactivation was conducted with light source with different light intensity and major emission wavelength. As expected, the light intensity greatly influences the inactivation efficiency of bacterial cell (Fig. 4a). A significant inactivation was observed when the light intensity was 200 mW cm^{-2}. However, when the light intensity decreased, the inactivation efficiency also decreased drastically. Only 1-log inactivation was achieved when the light intensity was decreased to 50 mW cm^{-2}. The difference between the bacterial cell density under different light intensity are statistically significant (one-way ANOVA, $P < 0.05$) Furthermore, the color of light (i.e. the major emission wavelength) also greatly influenced the inactivation efficiency. With the same light intensity (6 mW cm^{-2}), only blue (455 nm) and white light enhanced the bacterial inactivation by H_2O_2 Little inactivation was obtained when red (633 nm) or green (520 nm) light was applied (Fig. 4b). The bacterial cell density under the blue and white light are statistically different from that of red and green light (one-way ANOVA, $p < 0.05$).

Since no production of ·OH or $\cdot O_2^-$ was detected during the process, therefore enhancement by light irradiation are possibly due to the change in physiology of bacterial cells. However, there are only a few studies which have investigated the effect of emission wavelength of visible light on the physiology of bacteria. Matallana-Surget et al. [28] reported that light spectrum of solar irradiation showed great impact on the physiological changes of a marine bacterium Sphingopyxis alaskensis. Moreover, low intensity of visible light would cause biochemical and morphological changes in *E. coli* [29,30]. Therefore, it is possible that only blue light trigger the physiological changes of *E. coli* which would increase their susceptibility toward H_2O_2. For the enhancement effect of white light LED lamp, it is because white light also comprises blue light and can therefore also improve the inactivation with a lower magnitude.

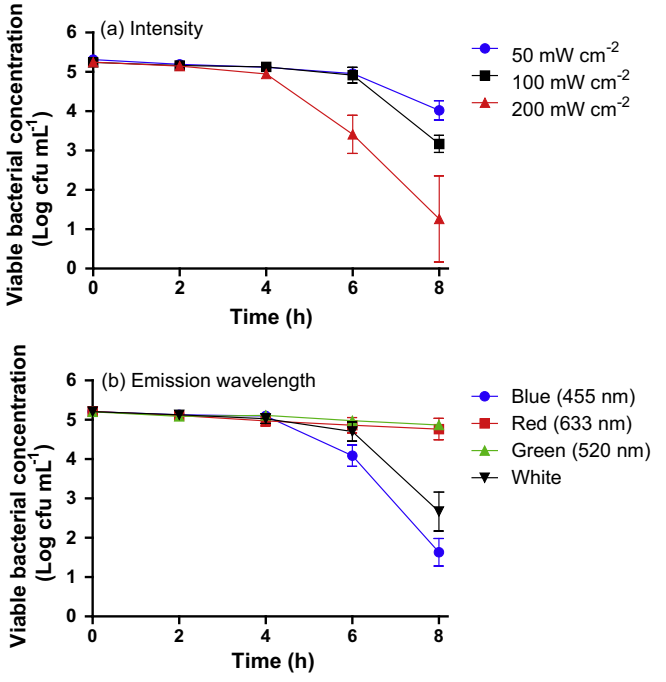

Fig. 4. Inactivation of *Escherichia coli* K-12 with continuous H_2O_2 supply in (a) different light intensity and (b) different light color (major emission wavelength). Initial *E. coli* K-12 concentration = 2×10^5 CFU mL^{-1}; H_2O_2 concentration = 400 μM; Detection limit of *E. coli* K-12 = 1 CFU mL^{-1}; Light source for study different light intensity = a Xenon lamp with UV-filter ($\lambda < 400$ nm). Light source for study of different color = a LED lamp with intensity of 6 mW cm^{-2}. The error bars represent the standard deviation of replicates ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Effect of light pretreatment on E. coli K-12

In the previous experiment, no generation of ·OH or $\cdot O_2^-$ were detected by the chemical probes. Therefore, the photolysis transformation of H_2O_2 into other radicals under light irradiation (e.g. UV can convert H_2O_2 into ·OH) [24,31] is not possible. Therefore, the effect of light irradiation is very likely due to the possible physiological changes of bacterial cells caused by light irradiation and the subsequent alteration of their susceptibility to H_2O_2.

To further confirm the enhancement effect on the inactivation with light irradiation is due to the physiology changes of bacterial cells instead of photolysis of H_2O_2, the effect of light pretreatment was also investigated. Fig. 5 shows that light-pretreatment of bacterial cells of *E. coli* K-12 can enhance the inactivation. The inactivation of *E. coli* K-12 with 1 h light pretreatment is comparable to the results of bacterial inactivation by 2 mM H_2O_2 in Fig. 3. In the dark control, no inactivation was observed. When light pretreatment was applied to the *E. coli* K-12, the inactivation efficiency was increased remarkably even the light irradiation was applied to the bacterial cells before the addition of H_2O_2. Also the enhancement was proportional to the duration of light pretreatment and the bacterial density among different light pretreatment duration are statistically different from each other (one-way ANOVA, $p < 0.05$). Because of the absence of light irradiation on the H_2O_2 during the inactivation process, the conversion of H_2O_2 into other ROSs by light irradiation is impossible in this experiment. The results suggested that light irradiation may alter the physiology of bacterial cells which increases their sensitivity to H_2O_2 instead of conversion the H_2O_2 into other radicals.

3.4. Effect of light pretreatment on different E. coli strains

Although the mechanism of H_2O_2 is still not completely elucidated, it is generally believed that H_2O_2 inactivates the bacterial cells by forming of strongly oxidant chemical species (i.e. ·OH)

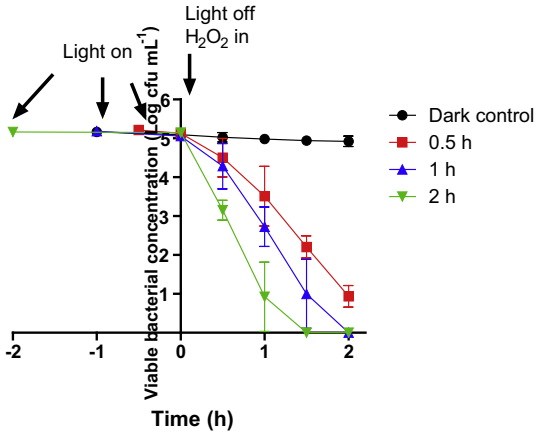

Fig. 5. Inactivation of *Escherichia coli* K-12 with light pretreatment in different pretreatment duration. Initial bacterial cells density = 2×10^5 CFU mL^{-1}; H_2O_2 concentration = 2 mM; Detection limit of *E. coli* K-12 = 1 CFU mL^{-1}; Pretreatment light source = a Xenon lamp with intensity of 200 mW cm^{-2}. The error bars represent the standard deviation of replicates ($n = 3$).

inside the bacterial cytoplasm instead of direct oxidizing the bacterial cells [10–12]. The synergistic effect between the light irradiation and H_2O_2 has been reported previously [31,32]. However, the underlying mechanism of the synergism has not been studied in detail.

To further investigate the effect of light irradiation, the inactivation of different *E. coli* mutants by H_2O_2 was conducted to identify the gene(s) related to the physiological change(s) of bacterial cell during the light exposure. According to the literature, the possible changes of the *E. coli* under light irradiation include, (1) suppression of DNA-binding protein, such as Dps (DNA-binding protein from starvation cells) [33], which lead to the increase of sensitivity of DNA to H_2O_2, and (2) alternation the iron regulation in bacterial cell, which lead to an increase in intracellular Fe^{2+}, thus increasing the ·OH production through Fenton's reaction [34]. Therefore, the inactivation efficiency of the parental strain (*E. coli* BW25113) and its single-gene deleted isogenic mutants, which are related to iron regulation (*fes*$^-$ and *entA*$^-$), DNA protection (*dps*$^-$) and catalase expression (*katE*$^-$), were investigated and compared with the light pretreatment. If the target gene is involved in the physiological changes of *E. coli* during light irradiation, the difference in inactivation efficiency of corresponding deletion mutants should be changed slightly or remained unchanged between the light pretreatment and dark control.

Similar to *E. coli* K-12, the light pretreatment enhances the inactivation of both the parental and mutant strains (Fig. 6). The inactivation efficiency of *E. coli* BW25113 is similar to that of *E. coli* K-12, suggesting that the derived *E. coli* BW25113 have similar susceptibility as *E. coli* K-12. However, the enhancement effect varied among different mutants. Complete inactivation was observed for the parental strain and *katE*$^-$ mutant within 2 h, and the *dps*$^-$ mutant was completely inactivated with 1.5 h, while 1-log and 3-log reduction for *entA*$^-$ and *fes*$^-$ mutants was observed, respectively. That is, only *fes*$^-$ and *entA*$^-$ mutants showed a higher resistance to H_2O_2 after light pretreatment compared to the parental strain. The difference in inactivation efficiency was very small between the light pretreatment and dark control for these two mutants (especially for *entA*$^-$ mutant), indicated these two genes involving in the physiological changes during light irradiation. The *fes* and *entA* are the genes responsible for the expression of enterochelin esterase and enterobactin which regulate the intracellular iron level [35]. *fes*$^-$ and *entA*$^-$ mutants have lower level of intracellular Fe^{2+}. Therefore, the catalytic conversion of H_2O_2 to ·OH by Fe^{2+} is slowed down, leading to a decrease in the inactivation efficiency. This result suggests that light irradiation up-regulates these two genes which increases the intracellular Fe^{2+} and favors the conversion of H_2O_2 to ·OH via catalytic reaction by Fe^{2+}, hence increasing the inactivation efficiency and intensifying the damage to DNA (see Fig. 6).

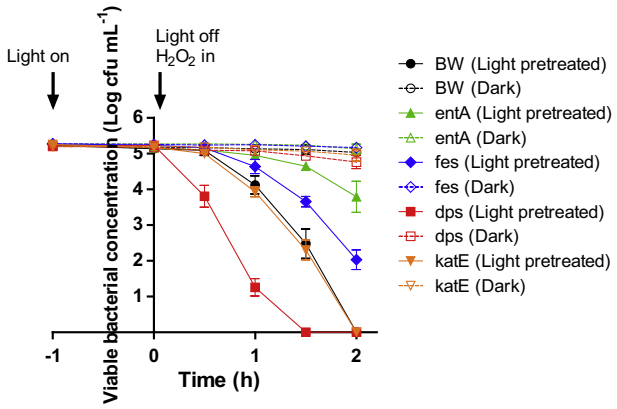

Fig. 6. Inactivation efficiency of *Escherichia coli* BW25113 and its isogenic single-gene deleted mutants with light pretreatment. Initial bacterial cells concentration = 2×10^5 CFU mL^{-1}; H_2O_2 concentration = 2 mM; pretreatment duration = 1 h; Detection limit of *E. coli* K-12 = 1 CFU mL^{-1}; Pretreatment light source = a Xenon lamp with intensity of 200 mW cm^{-2}. The error bars represent the standard deviation of replicates (n = 3).

The results of *dps*$^-$ mutant also supported the hypothesis that the inactivation of bacterial cell is due to the damage of the DNA. Dps has been reported for its protection of DNA from the oxidative stresses [36]. Therefore *dps*$^-$ mutant has a lower Dps level and leads to a lower degree of DNA protection, thus a higher sensitivity to ·OH (converted from H_2O_2 through the catalytic reaction by Fe^{2+}) after light pretreatment compared to the parental strain. Although KatE is an important enzyme involving in the H_2O_2 dismutation, the *katE*$^-$ mutant does not show any significant difference in inactivation efficiency compared with that of the parental strain. The results suggest that the decrease in catalase level is not related to the synergy between the H_2O_2 and light irradiation. A previous study also showed that light irradiation does not suppress catalase activities [33]. Moreover, since the inactivation of *dps*$^-$ mutant by H_2O_2 was faster than that of the *katE*$^-$ mutant, the results suggest that the protection of DNA by Dps is more significant than the removal of H_2O_2 by KatE for the resistance of H_2O_2 in bacterial cell.

Thus, based on the above mentioned experiments, the following mechanism of the synergistic effect between the H_2O_2 and light irradiation is proposed. Light irradiation would increase the intracellular Fe^{2+} and decrease the protection of the bacterial DNA which would favor the production of ·OH through the catalytic conversion of H_2O_2 by Fe^{2+} and increase the damage to DNA. The results show that iron regulation and DNA protection play an important role in the bacterial defense system against H_2O_2 in bacterial inactivation.

3.5. Environmental implication

The ·OH has been accepted as the major ROS that responsible for the photocatalytic bacterial inactivation. it attacks bacterial cell membrane and leads to irreversible damages [1]. However, some recent studies reported that other ROSs are also involve in photocatalytic bacterial inactivation [8,9]. Moreover, the production of ·OH was not detected in some visible-light-driven (VLD) photocatalytic system [3,9,22] and H_2O_2 even play a major role for the bacterial disinfection in some photocatalytic systems [3,27]. Therefore, this study provides addition information to support the importance of other ROSs (i.e. H_2O_2) in VLD photocatalytic bacterial inactivation. Furthermore, the results of this study also suggest that physiology of the bacterial cells has great influence on the efficiency of bacterial inactivation. Beside light irradiation, other factors would also affect the susceptibility of the bacterial cells in photocatalytic inactivation process. For example, an increase in temperature alters the fatty acid profile of the *E. coli* and changes the change of susceptibility of the bacterial cells in photocatalytic inactivation process [37]. Therefore, the impact of different physico-chemical factors on the bacterial cell during the bacterial inactivation process should also be considered.

Beside photocatalysis, solar disinfection (SODIS) is well-studied system for water disinfection. Recent studies showed that addition of low concentration of H_2O_2 during SODIS can enhance the bacterial inactivation efficiency [32]. Although, the existence of the synergism of light treatment and H_2O_2 on bacterial inactivation is demonstrated, its mechanism is seldom studied. Feuerstein et al. [31] reported that the synergic effect between blue light and H_2O_2 on inactivation of *Streptococcus* mutants is a photochemical process involving ROS. The results in this study enrich the interpretation of the synergism in the aspect of bacterial physiology. Besides converting H_2O_2 into more powerful ROS, sunlight irradiation would also change the physiology of bacterial cells which

enhance their susceptibility to H_2O_2, resulting in a more efficient bacterial disinfection.

In the natural aquatic environment, the presence of low concentration of H_2O_2 (100 nM) in natural water is very common [38]. According to the results of this study, this concentration of H_2O_2 should not be high enough to inactivate bacterial cells. However, the concentration of H_2O_2 in water may increase to 2–3 order after a rainfall [39]. In this case, the increase in concentration of H_2O_2 (i.e. after a rainfall) and with sunlight irradiation would have a great influence on the bacterial survival in the natural water system.

4. Conclusions

In general, scavenger addition study and measurement of the concentration of ROSs are the major strategies for showing the contribution of ROSs in photocatalytic inactivation. We used a modified partition system and inactivation with light pretreatment of different *E. coli* mutants as compared with the parental strain to reveal the importance of H_2O_2 and light irradiation in photocatalytic inactivation. H_2O_2 produced in photocatalysis, even in a continuous supply mode, is not effective for the inactivation of bacterial cells. Our results suggest that the properties of the light source significantly affect the bacterial inactivation efficiency of H_2O_2. For example, the inactivation is less effective with a light source at low intensity, such as the fluorescence tube and LED lamps, because the inactivation of the bacterial cells is proportional to the light intensity and dependent on major emission wavelength. Moreover, the need for standard procedures for testing the inactivation abilities of photocatalysts should be re-emphasized [40]. If the experimental conditions of photocatalytic inactivation such as light source is not standardized, it will be difficult to compare the photocatalytic inactivation abilities between different photocatalysts as light irradiation alter the physiology of bacterial cells which also affect the inactivation efficiency.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jphotobiol.2015.06.007.

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