Research Article

Photocatalysis disinfection of *Bacillus subtilis* spores in water under sunlight irradiation

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Abstract

This study investigated TiO_2 photocatalysis disinfection efficiency on spores of *B. subtilis* under black light, sunlight and indoor natural daylight with the aid of H_2O_2. It was found that disinfection efficiency from sunlight irradiation (UVA intensity of 5.93-18.2 W/m^2) was higher than that from black light irradiation (UVA intensity of 13.5 W/m^2) and indoor natural daylight (UVA intensity of 0 W/m^2), respectively. A 95.3% spore removal within 1 h occurred when sunlight UVA intensity reached 23.45 W/m^2 with the assistance of 80 mM H_2O_2. Between 9 am and 4 pm (min-max UVA intensity of 8.49-23.45 W/m^2), spore removal did not significantly vary ($p>0.05$).

Keywords: solar photocatalysis; spore removal; *B. subtilis*; water disinfection; TiO_2.

1. Introduction

Chlorination is the most popular method for water disinfection in many countries due to its easy usage and long residual efficiency. However, when organic matter is present, disinfection by-products such as trihalomethanes and haloacetic acids which are carcinogens and mutagens can be generated [1-3]. Furthermore, chlorine cannot destroy *Bacillus* spp. which are gram positive bacteria that form endospores [4], nor *Cryptosporidium parvum* and *Giardia lamblia* which are protozoa that cause life-threatening diarrhea [5], nor other microorganisms that generate biofilm [6]. Therefore, chlorination is neither effective nor safe and needs to be replaced by more reliable methods.

One promising technology developed in this decade is photocatalysis that produces free radicals. The process needs a semiconductor metal as the catalyst, of which the most popular is titanium dioxide (TiO_2). When TiO_2 is irradiated with light that has photon energy greater than its band gap energy (≤ 365 nm), electrons in the valence band can absorb that energy and jump to the conduction band, leaving holes (h^+) behind. These holes can react with hydroxides in water to produce hydroxyl radicals (°OH), while electrons in the conduction band can react with oxygen to become superoxide radical anions ($°O_2^-$). Both radicals have strong oxidizing power, thus they can destroy microorganism cell

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walls, releasing the internal organelles and causing the cells to die [7].

Most researchers have used black light lamps as the light source to provoke photocatalytic reactions. For example, Ibáñez et al. [8] used black light lamps with a UVA intensity of 5.5 mW/cm^2 irradiating onto 0.1 g/L TiO_2 powder to eliminate *Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella typhimurium*. The reactions destroyed 99.9% of bacteria within 40 min. Another example is Paleologou et al. [3] who used 9-watt black light lamps irradiating onto 0.1-0.75 g/L TiO_2 powder mixed with 25-100 mg/L hydrogen peroxide (H_2O_2) to inactivate 10^4-10^5 cfu/ml *E. coli*. Within 20 min, almost all bacteria were killed. Other groups have used a solar simulating lamp to mimic solar irradiation. For example, Seven et al. [9] used a 400-watt sodium lamp for solar simulation irradiating onto 0.01 mg/mL TiO_2 powder for 4 hr to eliminate *E. coli, P. aeruginosa, Staphylococcus aureus, Saccharomyces cerevisiae, Candida albicans*, and *Aspergillus niger* in water. They found that within 40 min, complete inactivation occurred for *E. coli* and *P. aeruginosa*, while that for *S. aureus, C. albicans* and *S. cerevisiae* required 120 min. Only *A. niger* was not destroyed by the photocatalysis since its cell envelope is more complex and much more resistant. Lonnen et al. [10] also used a 1000-watt xenon arc solar simulating lamp irradiating onto 25 mg/cm^2 TiO_2 immobilized onto transparent plastic sheet to reduce concentrations of *Acanthamoeba polyphaga, Candida albicans, Fusarium solani, Pseudomonas aeruginosa, E. coli* and *Bacillus subtilis* spores in water. They found that *A. polyphaga* (trophozoite stage), *C. albicans, F. solani, P. aeruginosa* and *E. coli* were reduced at least 4 log units within 8 hr, whereas spores of *B. subtilis* that were reduced only 1.7 log units. However, the cyst stage of *A. polyphaga* could not be destroyed at all by the reactions.

From these reviews, some uncertainties remain about solar photocatalysis performance, for instance, whether solar photocatalysis is capable of eliminating resistant forms such as spores of microorganisms. *Bacillus subtilis* spores could endure the harsh environment 9-fold better than its vegetative cell or than *E. coli* [11]. In addition, most available information is from solar simulating lamps set up in well-controlled laboratories, not from real situations. If sunlight is applied to the photocatalytic reactions, it is not clear whether its capability will be the same as the reports in the literature. This study therefore explores the photocatalysis of *Bacillus subtilis* spores with the aid of H_2O_2 under sunlight irradiation to answer these questions. Thailand is located in the tropical zone which has plenty of sunlight all year round. If solar photocatalysis from sunlight is successful in water disinfection, the benefits would be substantial for many other countries that have similar weather patterns.

2. Materials and Methods

2.1 TiO_2 immobilizing

In this study, titanium dioxide (Degussa P-25) was immobilized onto a glass plate instead of using it in a powder form. Five grams of TiO_2 were dissolved in 100 mL of 99.9% ethanol, and 3.5 mL of alkoxysilane was added to the slurry as a binder. The suspension was stirred at 500 rpm for 30 min. Then an 8-cm wide×20-cm long×2-mm thick glass plate was dipped into the suspension for 5 min and pulled out by machine at the rate of 0.25 mm/sec. The plate was dried at room temperature and re-dipped for 3 rounds, then baked at 400°C for 1 h to remove the residual organic compounds. Average TiO_2 loading on the glass plate was around 160 mg/cm^2.

2.2 *Bacillus subtilis* spore preparation

B. subtilis (TISTR008) was purchased in freeze dried culture from the Microbiological Resources Centre (MIRCEN), Thailand Institute of Scientific and Technological Research. To revive the microorganism, the culture was suspended in sterile water and transferred onto Tryptic Soy Agar (TSA) plates (Difco, USA) and incubated at 37 °C for 7 days until the spores were formed. A few loops of spores were mixed with sterile water and boiled at 80 °C for 10 min to destroy the vegetative cells and then centrifuged at 2500 rpm (Hettich, model Rotofix 32) for 15 min. The spores in the supernatant were used in the experiment.

2.3 Photocatalysis disinfection experiment

Two coated-glass plates were placed at the walls of an 8-cm wide×20-cm long×22-cm high glass reactor, which was filled with 1 L of sterile distilled water mixed with *B. subtilis* spores. The initial concentration of *B. subtilis* spores was determined by withdrawing 0.1 mL of sample to spread onto the TSA plates and incubated at 37 °C for 12-24 h. Initial spore concentrations ranged from $1.2\text{-}2.3\times10^4$ cfu/mL. Hydrogen peroxide at 5-80 mM was added to the water in each reactor. The first set of reactors was placed under sunlight on the rooftop of a two-story building, starting from 9 am or 10 am for three hours. The UVA and UVB intensities were recorded every 30 min with a radiometer (Viber Lourmar, model VLX-3W). The second set was placed under three black light lamps (Phillips model TLD 36W/08) with a UVA intensity of 1.3 mW/cm^2 (13 W/m^2). The third set was placed in a laboratory during daytime under fluorescent light (UVA intensity of 0 mW/cm^2), which from this point forward is called "natural light." Every 30 min for a total of 180 min, 0.1 mL of sample was withdrawn from each reactor to measure the remaining *B. subtilis* concentration. The effectiveness of 5-80 mM H_2O_2 alone without coated-glass plates was simultaneously studied.

The relationship between UV intensity in sunlight and water disinfection in each hour was also investigated. The reactors were filled with coated-glass plates and 80 mM H_2O_2 and placed under sunlight for 1 h periods from 9 am till 4 pm. The water in the reactor was withdrawn every 15 min to observe the bacterial survival rates. Solar UVA and UVB were recorded every 15 min.

3. Results and Discussion

3.1 Natural decay of *B. subtilis* spores

The study started with an investigation of decay rates of *B. subtilis* spores under natural light compared with sunlight without photocatalysis. No TiO_2-coated glass plate nor H_2O_2 was added to the reactors. The results are shown in Figure 1 in the form of C/C_0 which is the concentration of *B. subtilis* at any time (C) divided by the initial concentration (C_0). The lower the number of C/C_0, the higher the efficiency.

It can be seen that radiation from sunlight alone could not destroy spores of *B. subtilis*; the reduction pattern was similar to the decay pattern in the absence of direct solar exposure. This matches the findings of Bandala et al. [12] who irradiated 1100 W/m^2 solar radiation (from a solar simulator) onto 10^7 cells/mL of *B. subtilis* spore suspension. They found that 12 hours of intense solar radiation (5500 kJ/L) were needed to reduce 1-log unit of *B. subtilis* spores. This is because spores of *B. subtilis* have a thick protein coat which is electron-dense at the outer layer and lamella-like at the inner layer. The inner coat layer is responsible for spore resistance to the UV radiation [13]. In addition, spores have a different photochemistry and DNA repair is more efficient under exposure to UVC

radiation (254 nm), thus spores of *B. subtilis* are 5-50 times more resistant than its vegetative cells in water [14].

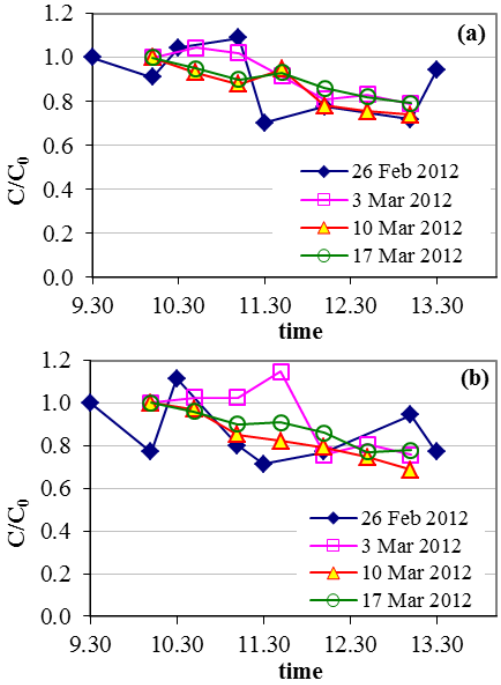
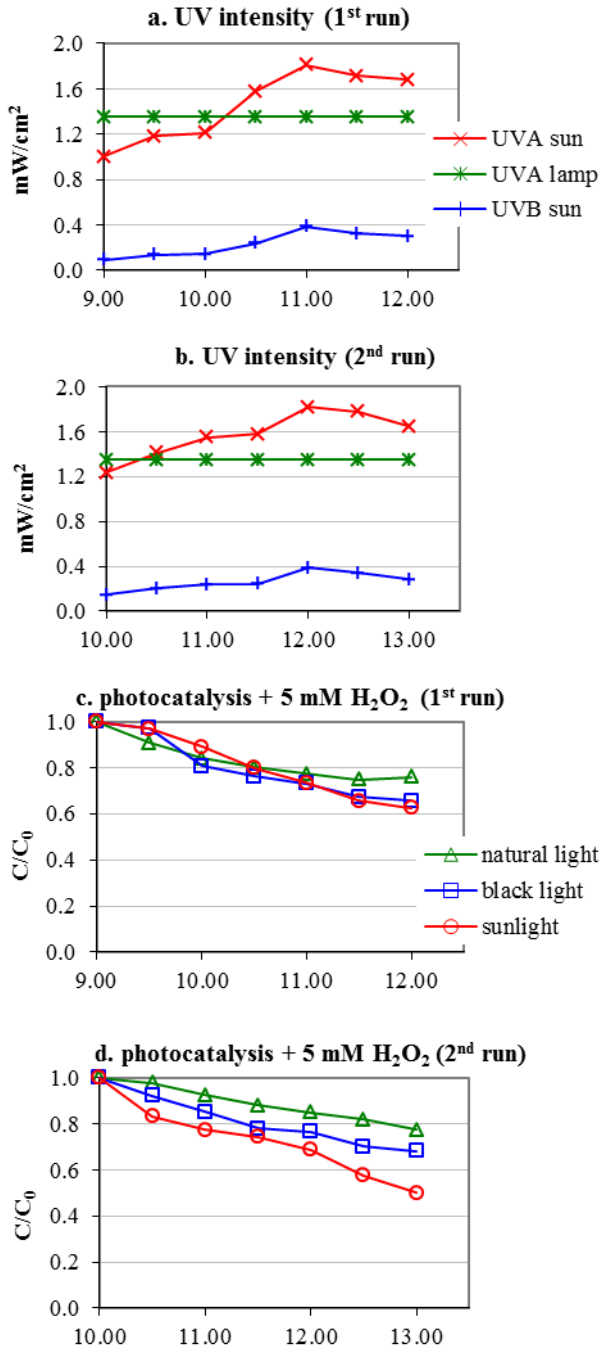


Fig.1. Reduction of *B. subtilis* spores from (a) natural decay and (b) from sunlight irradiation.

3.2 Photocatalysis from Black light, Sunlight, and Natural light

TiO_2-coated glass plates were placed in the reactors and spore suspensions were irradiated with different light sources in order to compare photocatalysis disinfection as shown in Figure 2. UVA and UVB irradiation intensities are also shown in Figures 2a and 2b. It should be noted that we attempted to control UVA intensity from black light lamp to equal to that from sunlight, but without success.



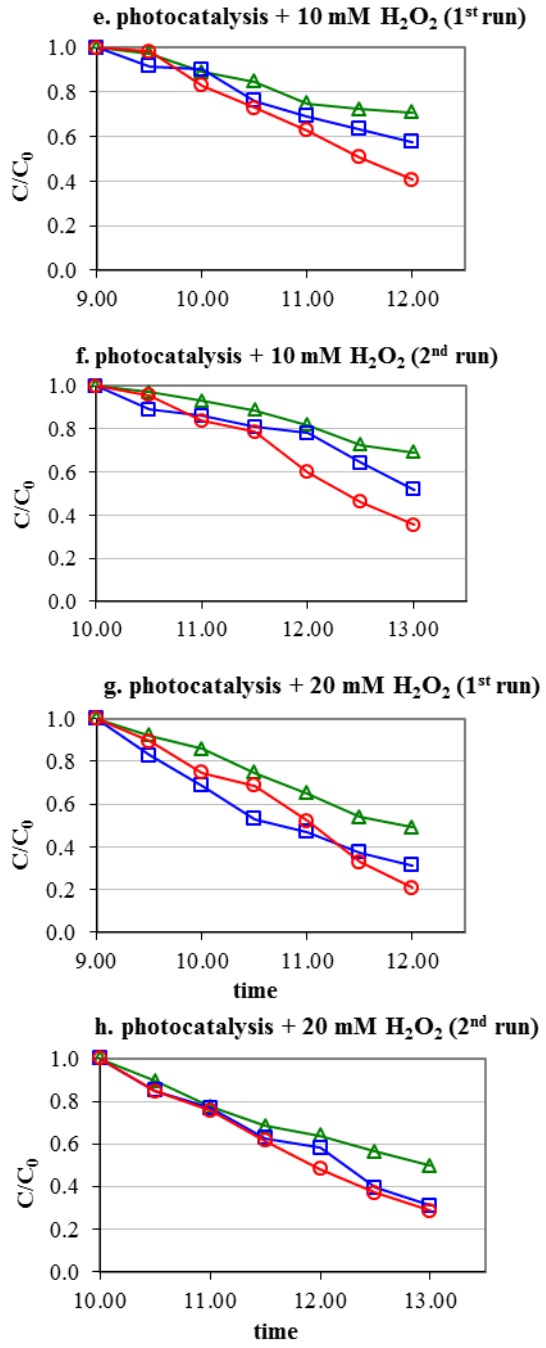


Fig.2. UV irradiation intensity and *B. subtilis* spore reduction from photocatalysis under different light sources, with the assistance of 5-20 mM H_2O_2

TiO_2 photocatalytic reactions start when TiO_2 absorbs photon energy from the light and creates electrons in the conduction band and holes in the valence band (eq.1). Holes and electrons further react with water and oxygen to generate free radicals (eq.2-3). H_2O_2 can act as an electron acceptor from photocatalytic reactions and generate more free radicals as shown in eq. 4-5 [15].

$$TiO_2 + h\upsilon \rightarrow TiO_2 (e^-_{cb} + h^+_{vb}) \quad (1)$$
$$h^+_{vb} + H_2O \rightarrow {}^\circ OH + H^+ \quad (2)$$
$$e^-_{cb} + O_2 \rightarrow O_2^{\circ -} \quad (3)$$
$$H_2O_2 + e^-_{cb} \rightarrow {}^\circ OH + OH^- \quad (4)$$
$$H_2O_2 + O2^{\circ -} \rightarrow {}^\circ OH + OH^- + O_2 \quad (5)$$

This could be the reason why H_2O_2 under natural light had a lower efficiency than under sunlight or black light. Under natural light, photon energy was not strong enough to create electrons and holes in these bands. Hence, fewer free radicals were produced. Nevertheless, because H_2O_2 can remove the protein coat of spores [16], greater rates of disinfection still occurred, compared to natural decay or solar irradiation alone. Since sunlight has stronger UVA and UVB intensities than black light, when H_2O_2 was added to the water photocatalysis from sunlight yielded the highest disinfection efficiency. Moreover, sunlight at wavelengths of less than 300 nm (spectrum of UVC) will generate more radicals due to eq.6 [17].

$$H_2O_2 + h\upsilon \rightarrow 2{}^\circ OH \quad (6)$$

This study did not monitor UVC intensity which may be the cause of the difference between each run shown in Figures 2c and 2d. It is possible that in Figure 2d, UVC might enhance the reactions by creating more hydroxyl radicals. However, with 20 mM H_2O_2 (Figures 2g and 2h), the difference between black light and sunlight photocatalysis was small. Third and fourth runs were performed, as shown in Figure 3.

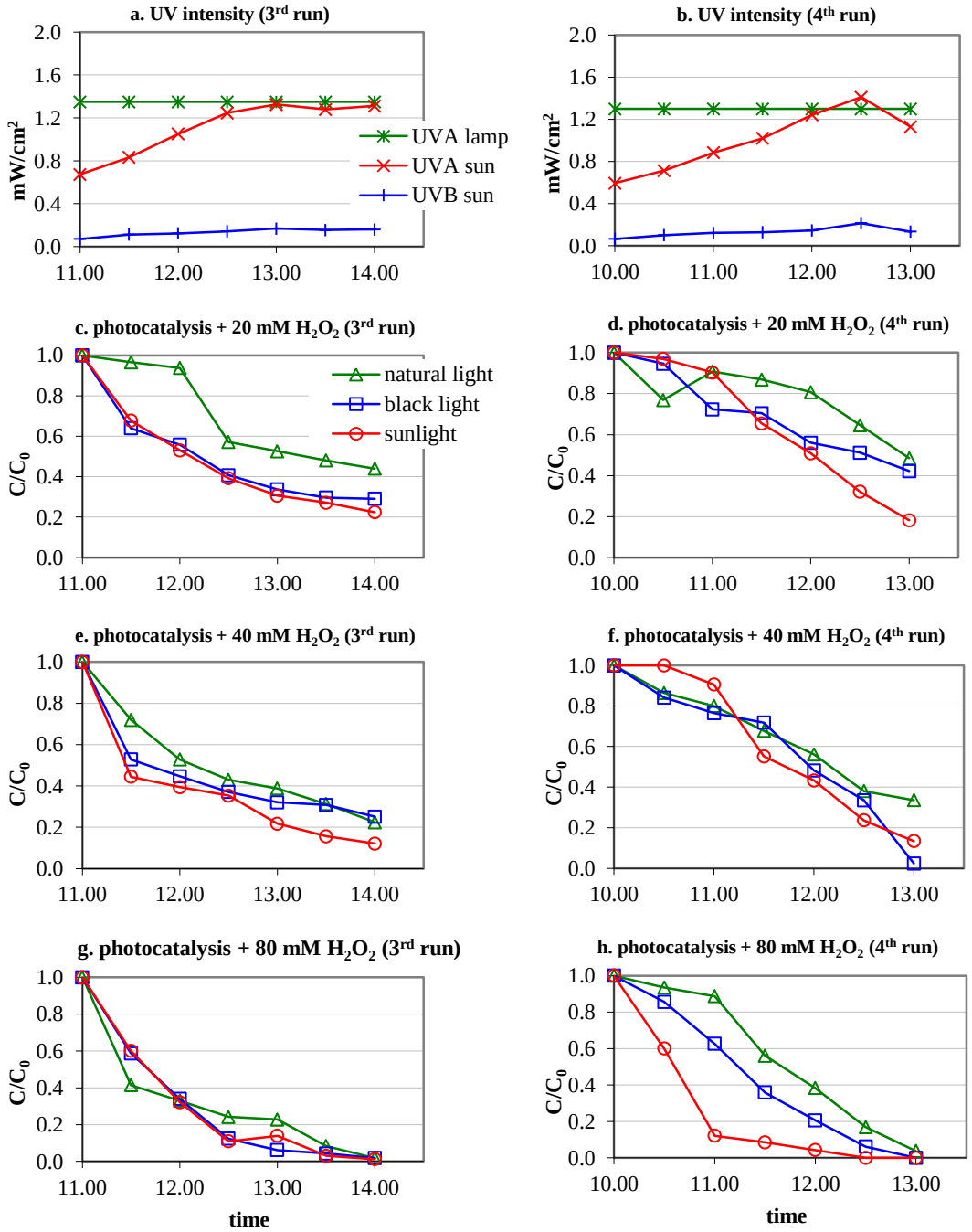
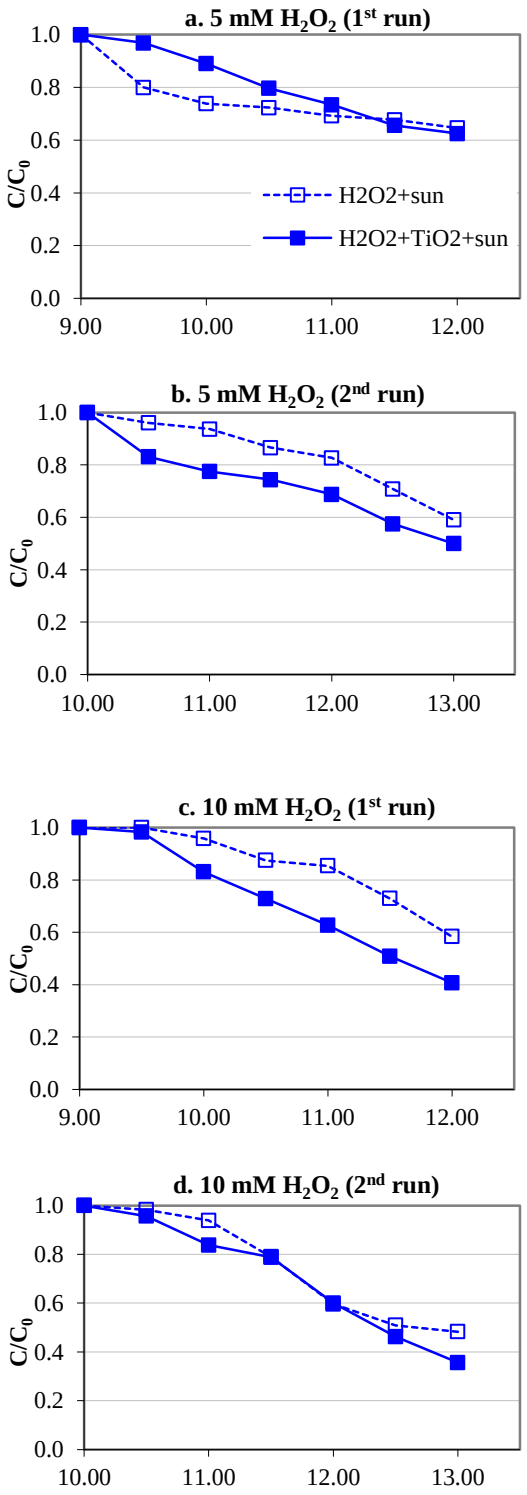


Fig.3. UVirradiation intensity and *B. subtilis* spore reduction from photocatalysis under different light sources, with the assistance of 20-80 mM H_2O_2.

UVA and UVB intensities in the third and fourth runs were about the same (Figures 3a and 3b), but the difference in disinfection efficiency between sunlight and black light was greater in the fourth run (Figure 3d) than in the third run (Figure 3c). Again, UVC intensity probably accounts for the difference. An alternative explanation is that each reactor might contain a different genotype of *B. subtilis* spore with different UV resistance, as reported in terms of LD_{90} (lethal dose for 90% killing). For example, LD_{90} for wild-type, *urvB*, *splB*, and *uvrB splB* are 300, 250, 110, and 7 J/m^2, respectively [14]. This means that spores can endure different UV intensities, depending on their genotypes. Thus, each reactor may show different results under the same UV composition and intensity. However, when H_2O_2 concentration was increased, better efficiency was observed. Almost 100% reduction occurred with 80 mM H_2O_2 within a 3-h exposure. This was superior to the results reported by Lonnen et al. [10] with a reduction of only 1.7-log unit of *B. subtilis* spores after 8 hours using a solar stimulating lamp (200 W/m^2 for 300-400 nm) irradiated onto 25 mg/cm^2 TiO_2 immobilized on plastic sheet. The maximum solar-UV intensity in the present study was around 18 W/m^2 and TiO_2 concentration was around 160 mg/cm^2. With the assistance of H_2O_2 and higher concentrations of TiO_2, more spore disinfection resulted.

3.3 Photocatalysis + H_2O_2 vs. H_2O_2 alone

Since both photocatalysis and H_2O_2 under solar irradiation can generate free radicals (eq.1-6), it was unclear which one was responsible for disinfection. A comparison was then made and the results are shown in Figure 4.



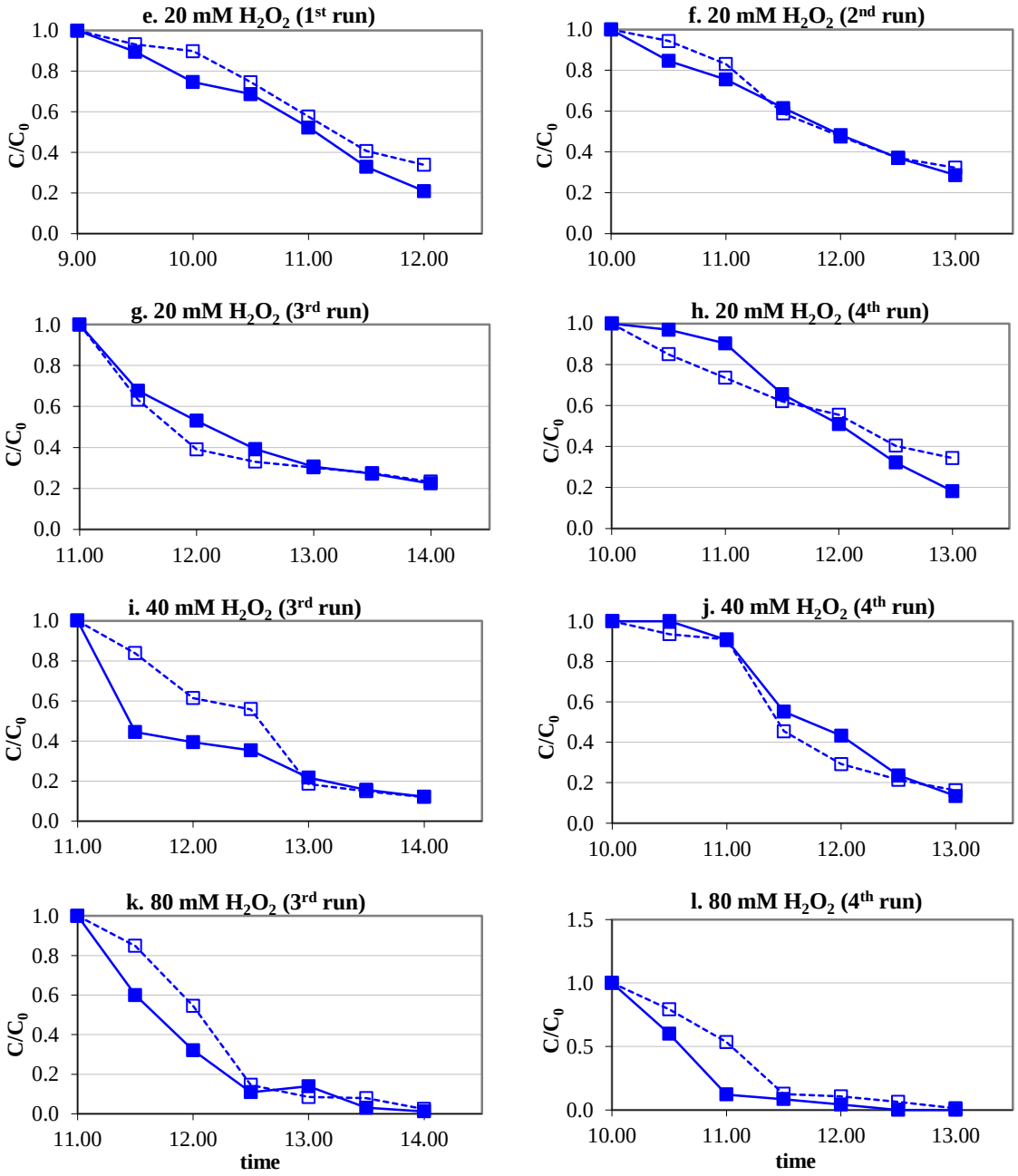


Fig.4. Reduction of *B. subtilis* spores from 5-80 mM H_2O_2 alone and H_2O_2 plus TiO_2 under sunlight irradiation.

The results seemed to show that H_2O_2 plus TiO_2 photocatalysis yields higher disinfection efficiency from synergistic effects than H_2O_2 alone. However, this was not always the case. It is possible that H_2O_2 might adsorp onto the TiO_2 surface and react with holes in the valence band to generate oxygen instead of free radicals (eq.7). Also H_2O_2 can decompose hydroxyl radicals to perhydroxyl (HOO°) (eq.8) which has weaker oxidizing potential [17].

$$H_2O_2 + 2\,h^+_{vb} \rightarrow O_2 + 2H^+ \quad (7)$$

$$H_2O_2 + {}^\circ OH \rightarrow H_2O + HOO^\circ \quad (8)$$

Malato et al. [17] have reported that if the pollutant concentration is too low and H_2O_2 concentration is too high, adsorption of H_2O_2 will occur and the reaction rate will decrease.

3.4 Solar-UV intensity vs. disinfection efficiency

Solar-UV intensity and the solar photocatalysis in each hour of a day were investigated. The results can be seen in Figures 5 and 6.

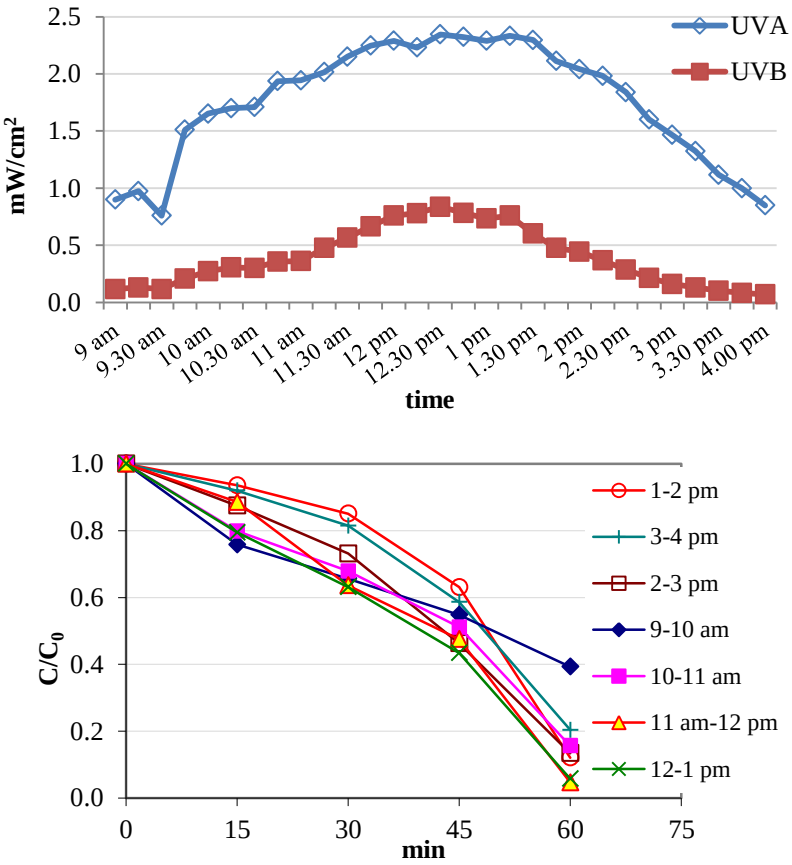


Fig.6. Photocatalysis disinfection in each hour.

It can be observed that the solar-UVA intensity was highest from noon to 1 pm (2.345mW/cm^2 or 23.45 W/m^2) and maximum spore disinfection (95.3%) was recorded in that period. This is similar to the results of Sichel et al. [18] who recorded an average solar-UVA irradiance of 25.75 W/m^2 in Spain. Almost complete inactivation of *Fusarium* spp. was found within 1-6 hours (depending on species). However, in the present study the UV intensity at 11 am-noon was close to that at 1-2 pm, but the disinfection efficiency between the two periods was the widest recorded, though still statistically insignificant (p=0.062) in paired t-tests. It can be seen that during almost the entire daylight period (from 9 am till 4 pm), natural solar photocatalysis was very effective for *B. subtilis* spore disinfection with no differences between hourly intervals (p=0.988) from ANOVA tests. Thus it is not necessary to perform solar-photocatalysis at peak intensity only.

4. Conclusions

It is clear that TiO_2 photocatalysis under sunlight irradiation is successful in spore disinfection. Although natural sunlight has limited UV intensities, if chemical oxidants such as H_2O_2 are added, enhancement of the photocatalytic reactions is clearly observed. Field research on large scale water disinfection by natural solar photocatalysis should thus be conducted in tropical countries.

5. Acknowledgements

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6. References

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