Ultraviolet A and B wavelength-dependent inactivation of viruses and bacteria in the water

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ABSTRACT

UVA and UVB can be applied to solar disinfection of water. In this study, the inactivation and photoreactivation of viruses and bacteria in the UVA-B range were analyzed. MS2 and T4 bacteriophages, and *Escherichia coli* were used as surrogates to quantify dose-response behaviors. Inactivation in UVC was used to validate the methodology and to expand the inactivation action spectra. The results showed log-linear inactivation for MS2 and T4 in the 254–320 nm wavelength range. T4 inactivation was consistently faster than MS2 (except at 320 nm), and for both phages, inactivation decreased with increasing wavelength. The dose-response of bacteria exhibited a lag at low doses, possibly because the photons must strike a discrete number of critical targets before growth stops. A tail was present at high doses for some wavelengths, perhaps due to clumping or the presence of subgroups with higher resistance. The inactivation action spectra for bacteria exhibited a reduction in inactivation as wavelength increased. No bacterial inactivation was observed beyond 320 nm at doses applied. After inactivation at 297 nm (UVA), bacteria regained viability through photoreactivation, and repair increased with increase in photoreactivating light exposure time. This implies additional doses above inactivation thresholds are required to cause irreversible damage. These results are useful for designing solar disinfection systems.

Key words | drinking water, solar disinfection, solar UV

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INTRODUCTION

The inactivation of water pathogens using ultraviolet (UV) radiation is widely accomplished using low- and medium-pressure lamps (LP and MP) that generate monochromatic and polychromatic radiation, respectively (Zimmer & Slawson 2002). UV sources with a wavelength band centered at approximately 254 nm (UVC) are the most commonly used in water treatment and are effective in inactivating microorganisms believed to cause a major safety concern in drinking water (Hijnen *et al.* 2006). Jagger (1967) indicated that the inactivation dose required for comparable levels of inactivation at 400, 340, and 300 nm are nominally 10^4, 10^3, 10 times higher, respectively, than that required at 260 nm. Other emerging UV sources include UV light-emitting diodes (LEDs), which provide some advantages over conventional sources, including flexible form factor, instant on/off behavior, and lack of mercury (Chatterley & Linden 2010; Bowker *et al.* 2011). The toxicity of mercury in MP and LP lamps is a potential health risk in the case where the lamp breaks (Würtele *et al.* 2011).

Disinfection systems based on conventional UV sources can be costly and out of reach of low-income communities in developing countries and disaster areas. Also, people deployed in remote or inaccessible areas (for instance, military and humanitarian agents) and without electricity cannot use these systems. Under these circumstances, solar disinfection (SODIS) of drinking water can represent a viable treatment option (Meierhofer & Landolt 2009). Coincidently, solar radiation tends to be intense and

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abundant in geographic locations where the majority of low-income communities, who do not have improved water treatment systems, are located; between the tropics, approximately 35°N–35°S (Sachs 2001; WHO/UNICEF 2004; Mbonimpa 2010). Solar radiation as a source of UVA and UVB is renewable, low cost, and avoids potential mercury contamination associated with lamps. However, solar radiation wavelengths are typically between 290 and 400 nm, with limited overlap with the most effective germicidal range (i.e., 200–300 nm); this means that SODIS applications may involve larger UV doses than those required for conventional UV disinfection systems that rely on artificial sources of UV radiation.

The lowest wavelength cutoff for ambient solar radiation varies spatially and temporally (Gueymard 2001; Duffie & Beckman 2006; Mbonimpa *et al.* 2012); as such, the performance of SODIS systems will display similar spatial and temporal dependence. The SMARTS model developed by Gueymard (1995) indicates that clear skies near the equator can yield radiation of wavelengths as short as 290–300 nm.

The development of SODIS technology has involved empirical methods to demonstrate inactivation of common pathogens in water contained in polyethylene terephthalate (PET) bottles exposed to solar radiation for at least 6 hours (Acra *et al.* 1984; McGuigan *et al.* 1998; Berney *et al.* 2006). The capabilities of SODIS, as any other UV inactivation technology, are commonly evaluated using the dose-response behavior, a method used to characterize reductions in viable or infective microbial concentration with respect to irradiation dose (Ubomba-Jaswa *et al.* 2009). SODIS systems have demonstrated a 3–4 $\log_{10}$ inactivation of *Escherichia coli, Vibrio cholerae*, *Salmonella, Shigella, Rotavirus*, and *Giardia*, and a 2–3 $\log_{10}$ inactivation for *Cryptosporidium* after a 6-hour solar exposure at geographic locations between 35°N–35°S (Meierhofer 2006). Similar capabilities of SODIS were also reported by Oates *et al.* (2003) for solar radiation intensity of about 500 W/m^2 and 5 hours of exposure. Laboratory-setting experiments using solar simulators indicated approximately a 6–6.5 $\log_{10}$ inactivation of *Vibrio, Shigella*, and *Salmonella*, with a 6–7 hour exposure to the intensity of about 2,400 kJ/m^2 for a 350–400 nm radiation (Berney *et al.* 2006). Heaselgrave *et al.* (2006) observed a 4.3 $\log_{10}$ inactivation of *poliovirus* with the intensity of about 850 W/m^2 from 320 to 700 nm radiation. For protozoan parasites, due to their ability to form protective oocysts and cysts (e.g., *C. parvum* and *Giardia*), McGuigan *et al.* (2006) indicated non-infectivity to mice when these microorganisms were exposed to 10 hours of solar radiation of about 870 W/m^2 and a cutoff at 320 nm. At wavelengths above 320 nm, a combined effect of UV and water temperature (above 45 °C) caused by solar radiation to inactivate pathogenic microorganisms was reported (McGuigan *et al.* 1998).

Controlling the efficiency of SODIS systems can be difficult because it will depend on the absorbance properties of the container, water turbidity, atmospheric conditions, and water mixing. For these reasons, SODIS may sometimes not meet US safe drinking water standards (EPA 2018). However, the SODIS system has made significant contributions to health outcomes in parts of the world that often lack access to potable drinking water. For example, a study found that the application of SODIS reduced both diarrhea (16–24%) and cholera (86%) in Kenyan children who drank water filled in PET plastic bottles exposed to sunlight for a day (Conroy *et al.* 2001; Meierhofer 2006; Graf *et al.* 2010). Improving the understanding of SODIS may help expand its applications and its associated health benefits.

The inactivation mechanisms associated with UVC exposure include the formation of cyclobutane pyrimidine dimers and pyrimidine-pyrimidone photoproducts (6-4PPs) in the DNA or RNA of microbial cells, which prevent replication and multiplication (Blatchley & Peel 2001). With these mechanisms highly reduced at longer UV wavelengths (i.e., UVB and UVA), other mechanisms of inactivation have been reported. Dejung *et al.* (2007) indicated damage to chromophores and their prosthetic groups called photosensitizers (FAD, NAD, heme, quinones, poryphrins, and Fe-scores). Kehoe *et al.* (2004) reported the formation of reactive oxidants, such as oxygen radicals and peroxides, caused by exogenous photosensitizers (e.g., humic substances) in water. Vidal & Diaz (2000) reported impairment of microbial cell membrane transport and catalase enzyme system leading to *E. coli* inactivation.

Photoreactivation represents a family of processes that facilitate repair and regrowth of cells that were previously inactivated by UV radiation. Jagger & Latarjet (1956) reported that repair can be caused by radiation between 313 and 549 nm. Jagger & Stafford (1965) showed how

E. coli B phr-, which was thought to be not reactivable under certain conditions, exhibited photorepair when the bacteria was in the log-growth phase and treated with a photoreactivating radiation at a wavelength of 334 nm. They also indicated photoprotection behavior when photoreactivating radiation was applied before inactivation. The majority of existing repair studies have been examined after UVC-induced inactivation. For example, Quek & Hu (2008b) observed reactivation of up to 80% after a 5 $\log_{10}$ inactivation of *E. coli* ATCC 11597 when low-pressure and medium-pressure lamps were used. Zimmer & Slawson (2002) indicated that repair occurred after inactivation using both medium-pressure (MP) and low-pressure (LP) lamps depending upon the irradiation dose. At doses higher than 3 mJ/cm^2 using a MP lamp, *E. coli* results did not show any repair. At 60 mJ/cm^2 for MP and LP, *E. coli* results also did not show any repair (Quek & Hu 2008a).

To date, most investigations of microbial dose-response behavior for SODIS systems have been conducted using solar simulators or ambient solar radiation at relevant locations. Many of these earlier works have not controlled for temporal or spatial variations of the applied UV spectrum. Therefore, some of these reported dose-response behaviors may not be generally applicable. Furthermore, few studies have involved investigations of photoreactivation after solar UV exposure. The objective of this study was to close some of these information gaps by quantifying wavelength-dependent dose-response behaviors for UV wavelengths that characterize the solar spectrum available on the Earth's surface using common bacterial and viral indicator species. Also examined in this study was photoreactivation after solar UV inactivation.

MATERIALS AND METHODS

UV source

The UV source was an ORIEL instrument (Newport Inc.) fitted with a 10 W medium-pressure mercury lamp, which provides an output spectrum with wavelengths ranging from 280 to 460 nm. Optical filters (Andover Corporation) were used to isolate narrow wavelength bands on the wavelength spectrum. The transmittance spectra of this series of optical filters are illustrated in Figure 1. The transmittance spectra of these filters were measured using a UV-Visible spectrometer (Varian, Cary 300 BIO). These filters were characterized by (nominal) half-height band widths of 10 nm and were identified with the wavelength corresponding to the peak of their respective transmittance spectra. The peak transmittance wavelengths for these filters were spaced at roughly 10 nm increments across the UVA and UVB range.

A conventional UV low-pressure mercury lamp, with an essentially monochromatic output ($\lambda = 254$ nm) and a XeBr excimer lamp ($\lambda = 282$ nm) were also used. These sources were both housed in flat-plate collimators (Blatchley 1997) which allowed delivery of collimated, monochromatic UV radiation that was quantifiable (in terms of incident irradiance) by the use of a radiometer (IL1700, International Light). Microbial dose-response behavior at these two wavelengths is well established; experiments conducted at these wavelengths were used as a benchmark for comparison with earlier work.

Exposure to UV

A Petri dish (polystyrene plastic) with a pure culture of microorganisms was used as a continuously mixed batch reactor (CMBR). The CMBR was placed under a collimated radiation beam; the free surface of the microbial suspension was perpendicular to the radiation beam and the Petri dish was uncovered to avoid absorbance of the lid (Figure S1, in Supplementary material, available with the online version of this paper). A magnetic stirrer was used to mix the microbial suspension. A batch system was used since it is difficult to determine photochemical reaction kinetics constants for continuous-flow systems (Blatchley 1997). The transmittance of the microbial suspension was measured

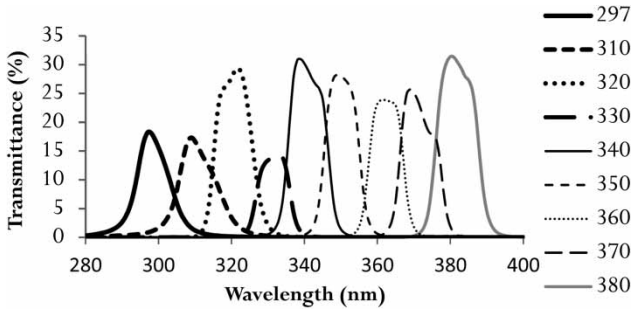

Figure 1 | Transmittance spectra of narrow bandpass optical filters used in this research.

using a UV-visible spectrometer (Varian, Cary 300 BIO). Also, at the interface of media: air and water, reflection and refraction were taken into account. The incident irradiance imposed on the liquid surface in the Petri dish was measured using a radiometer (IL1700, International Light). The average intensity (irradiance) in the mixed suspension can be determined mathematically from integration of the Beer–Lambert law (Bolton & Linden 2003; Mamane & Linden 2006). Sampling for each batch was done in triplicate. Details about the size of Petri dish, sample depth, calculation of average intensity, exposure times, and sampling frequency can be found in the Supplementary material (Tables S1–S4, available with the online version of this paper).

Surrogate microorganisms used

Dose-response experiments were performed using MS2 bacteriophage, a single-stranded RNA bacteriophage, in the family of *Leviviridae*, with a genome size of 3,569 nucleotides, and 24–26 nm in diameter. This phage is commonly considered as a surrogate for pathogenic human enteric viruses, widely used in experimentation to validate UV reactors, and is more resistant to UV exposure than many microbial pathogens (USEPA 2006; Fallon *et al.* 2007). Also, T4 bacteriophage was used here as a surrogate for DNA viruses. It is a double-stranded DNA bacteriophage in the family of *Myoviridae*, with a genome size of 336,000 nucleotides, a diameter of 65–80 nm, and a length of 120 nm. T4 is known to be more susceptible to UV at 254 nm than MS2 (Fallon *et al.* 2007). *E. coli* ATCC 15597 was used as a surrogate for bacterial pathogens and as a host for MS2. *E. coli* ATCC 11303 was used as a host for T4. Dark and light repair experiments were conducted to check the potential for bacterial repair after UVB exposure using *E. coli* ATCC 15597. It has been reported to have a high repair potential compared to the majority of *E. coli* strains (Quek & Hu 2008b). Details of the repair experiments are presented in the section 'Light and dark repair test'.

Bacteriophage analysis and detection using plaque assay method

Bacteriophage MS2 (ATCC 15597B1) was grown using *E. coli* (ATCC 15597) as a host, as follows:

1. Propagation of *E. coli*: An ampoule of *E. coli* (ATCC 15597) was rehydrated with 1 mL of tryptone-yeast extract (TYE) broth. The TYE broth contained 10 g/L of Tryptone, 1 g/L of yeast extract, and 8 g/L of sodium chloride in de-ionized water. This mixture was pre-sterilized using an autoclave (Napco model 8000-DSE). A few drops of suspension were inoculated on agar (TYE +15 g/L agar) plates and incubated at 37 °C for 24 hours.
2. Propagation of MS2: A 24-hr-old *E. coli* colony was removed from an agar plate, added to TYE broth, and incubated at 37 °C until the absorbance (600 nm) of the solution reached between 0.2 and 0.3 cm^{-1}. This took roughly 2–3 hours, and at this stage *E. coli* growth was assumed to be in log-phase. A sterile solution of Ca-glucose (1 g/L glucose, 3 g/L $CaCl_2$, and 10 mg/L thiamine) was added to the suspension to facilitate bacteriophage attachment to the host. A few drops of MS2 suspension from the ATCC ampoule were added to the actively growing *E. coli* and incubated at 37 °C for 24 hours. The suspension was filtered through a 0.22 μm membrane filter and stored at 4 °C. This suspension contained an MS2 concentration of roughly 10^{10} PFU/mL.
3. Plaque assay: Agar plates were prepared by pouring molten and sterile (autoclaved) agar into Petri dishes. Five serial dilutions of MS2 samples were prepared and all samples were analyzed in triplicate. 100 μL of the *E. coli* host cell suspension and 10 μL MS2 were added into 2.5 mL of soft-agar (TYE + 7.5 g/L agar). Soft-agar was overlaid on an agar plate, then allowed to solidify and incubated for 24 hours at 37 °C. Visible MS2 plaques were formed on the plates and counted. T4 was also enumerated using the top agar method with *E. coli* ATCC 11303 as host. Handling was otherwise similar to the methods used for MS2.

E. coli analysis

E. coli (ATCC 15597) was washed twice by centrifuging using sterile DI water to remove nutrient media, and resuspended in saline water (7% NaCl) before each dose-response experiment. Removal of the media was conducted to limit the potential for growth during the experiments. After exposure, *E. coli* was grown on agar (TYE + 15 g/L agar) in a Petri dish and enumeration was done by counting

colony forming units (CFUs). The concentration in the liquid phase was expressed in CFU/mL.

Light and dark repair test

The CMBRs were irradiated using a collimated beam device equipped with a narrow band filter with a peak at 297 nm, and sample solutions were completely stirred during exposure. For the first exposure, *E. coli* was subjected to a dose of 219 mJ/cm^2, resulting in a 5.8 log$_{10}$ inactivation. For the second exposure, *E. coli* was subjected to a dose of 314 mJ/cm^2 and a 6.06 log$_{10}$ inactivation was achieved. These doses were used to reach inactivation in the tailing region to test a hypothesis from the literature that repair may be eliminated beyond a threshold applied dose (Zimmer & Slawson 2002). Repair associated with lower doses has been reported in other studies (Quek & Hu 2008a), and the results were used in this study for comparison.

After UV irradiation, one batch of samples was exposed to radiation from an incandescent lamp (Sylvania, 60 W) as a source of repair light. The radiation spectrum for this lamp contains UVA and visible light (Figure 2), both of which are potentially important for photorepair. Another batch of samples was tested for dark repair by covering with aluminum foil. These solutions were sampled every hour and viable *E. coli* concentration determined.

Data analysis

The dose-response curves were built with the vertical axis (Y-axis) showing the log$_{10}$ of the ratio between microbial count after UV/light exposure (N) and the initial count before exposure (N$_o$). The dose of the X-axis is the product of average intensity (Irradiance) and exposure time. An action spectrum, defined as the relationship between the inactivation constant and wavelength (Jagger 1967) was generated. The inactivation constant is the inverse of a dose that causes 1 log inactivation (1/dose). For the repair study, viable *E. coli* was represented as a function of time. The data were compared with data from a repair study by Quek & Hu (2008a). The error bars indicate standard deviation around the mean. The dose-response data for bacteria and virus were fit, where appropriate, with various UV inactivation kinetics models, explained in previous studies (Severin *et al.* 1983; Pennell *et al.* 2008).

RESULTS

Dose-response behavior for viruses

MS2 was first analyzed at 254 nm to validate bacteriophage dose-response behavior against previously published results. The dose-response behavior of MS2 demonstrated log-linear behavior, consistent with a single-event (also known as single-hit or first-order) inactivation model for the range of doses applied ($R^2 = 0.97$). Linear regression of the log-transformed values of N/N$_0$ vs. dose yielded an inactivation constant estimate of 0.0561 cm^2/mJ (Figure 3). This inactivation behavior fell within the recommended upper and lower bounds of acceptable MS2 dose-response behavior, as defined by the USEPA (2006) (Figure 3).

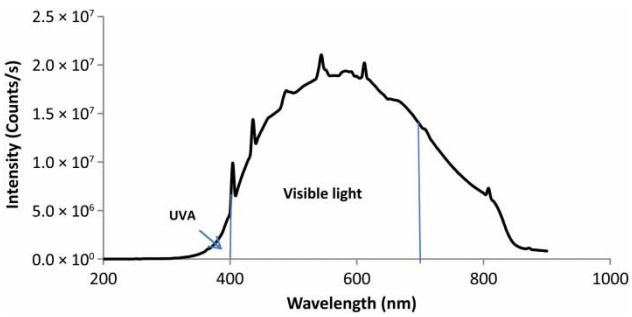

Figure 2 | Incadescent lamp output spectrum measured using a spectroflorometer (Horiba, FluoroLog-3). *Note:* Intensity units from the instrument are interpreted as arbitrary units or counts per second.

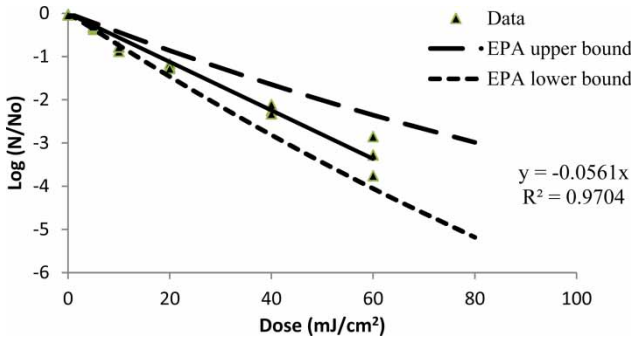

Figure 3 | Dose-response behavior for coliphage MS2 at 254 nm. Upper and lower bounds for UV$_{254}$ dose-response behavior of MS2, as defined by USEPA (2006) are included for reference.

Similar experiments were conducted at wavelengths of 282 nm, 297 nm, 310 nm, and 320 nm. Figure 4 presents a graphical summary of the data from all four wavelengths for MS2. At all five wavelengths, the dose-response behavior of MS2 conformed to a single-event model.

In a similar manner, data from dose-response experiments at three wavelengths are presented in Figure 4 for T4. As with MS2, the data from these experiments at all wavelengths conformed to the single-event model.

It should be noted that dose-response experiments were also conducted with MS2 and T4 at a wavelength of 330 nm, but no discernable inactivation response was observed for the range of UV doses applied. Therefore, dose-response behaviors for MS2 and T4 at 330 nm are not included in the figure.

Action spectra for MS2 and T4 for the wavelength range of 254–320 nm are illustrated in Figure 5. Inactivation constants for T4 at 254 nm and 282 nm were deduced from Fallon *et al.* (2007) and Winkler *et al.* (1962), respectively. Several distinct trends were evident in these data. First, T4 was generally more sensitive to UV irradiation than MS2. Second, both viruses demonstrated consistent decreases in inactivation response with increasing wavelength. An exception to this generalization was observed with MS2 at a wavelength of 320 nm. MS2 inactivation at 320 nm was slightly greater than at 310 nm. In general terms, microbial inactivation responses to UVC and UVB irradiation are attributable to photochemical damage to nucleic acids and proteins (Jagger 1967). Nucleic acids generally demonstrate a monotonic trend of decreasing absorbance with increasing wavelength above their absorbance peak, which generally is observed in the vicinity of 260 nm.

Dose-response behavior for bacteria

The UV-dose response behavior of *E. coli*, when exposed to 297 nm, showed a lag in the lower doses, and tailing (flattening at the lower end) at higher doses, with a first-order (log-linear) slope in between (Figure 6). The lag occurred below doses of about 10 mJ/cm^2, and tailing was observed at doses higher than approximately 100 mJ/cm^2 when the inactivation was about 6 log (99.9999% removal). For 310 nm

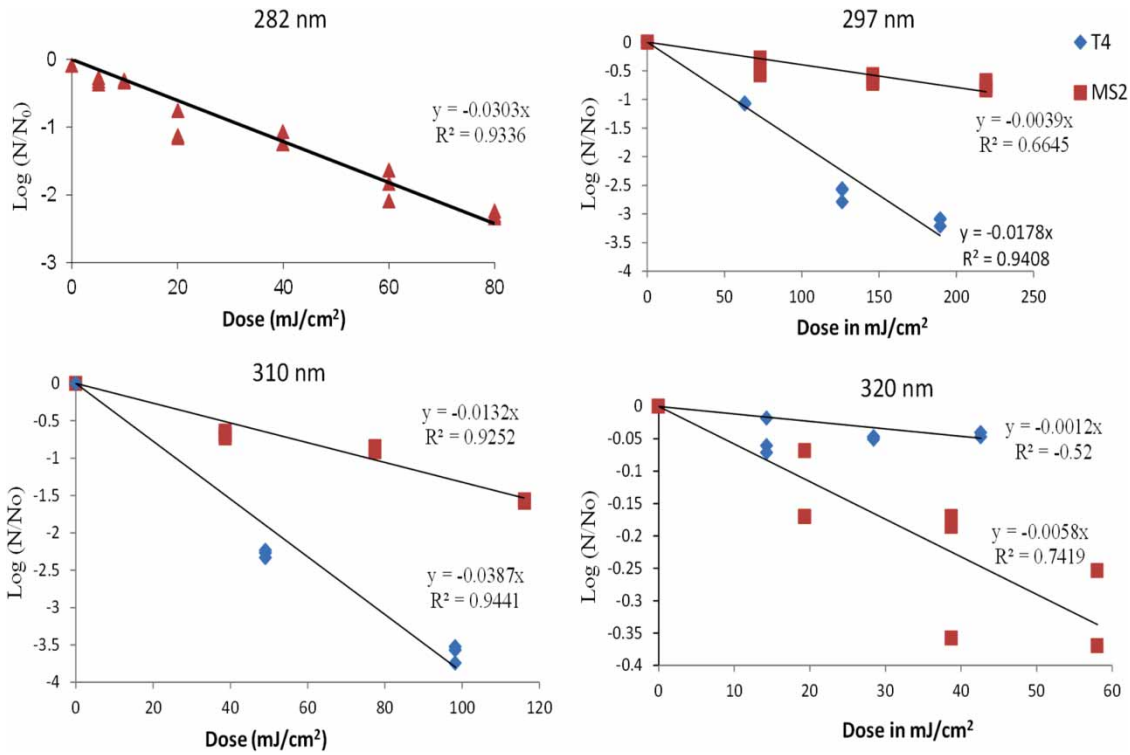

Figure 4 | Dose-response curve for MS2 at 282 nm and MS2 and T4 at 297 nm, 310 nm, and 320 nm.

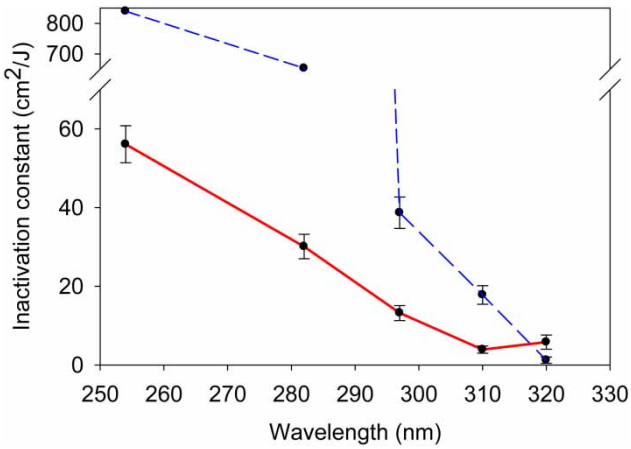

Figure 5 | Action spectra for MS2 (continous line), T4 (dashed line). T4 inactivation constants at 254 nm and 282 nm were deduced from Fallon *et al.* (2007) and Winkler *et al.* (1962), respectively.

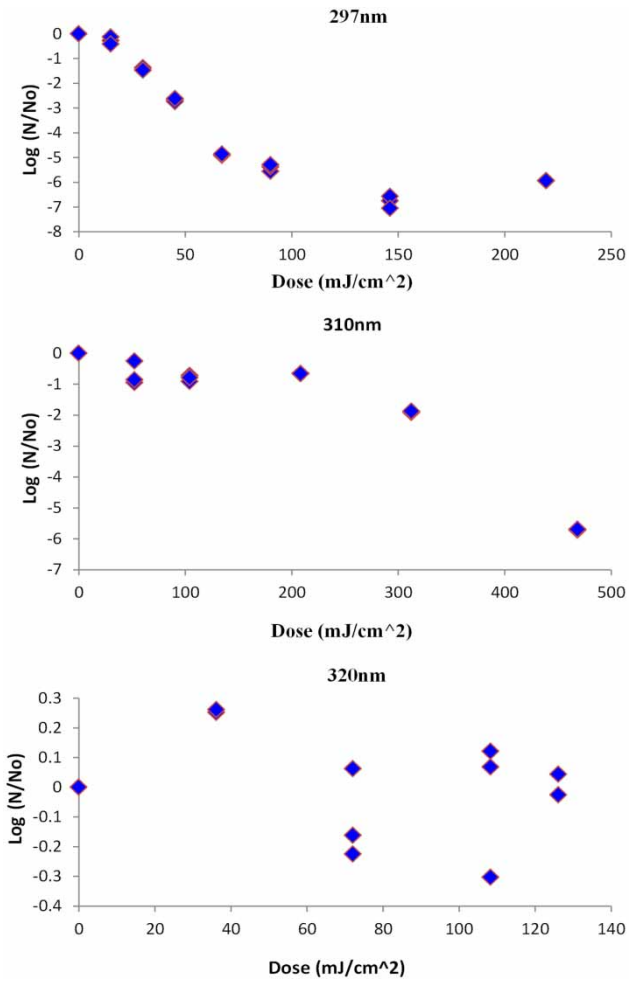

Figure 6 | Dose-response curve for *E. coli* at 297 nm, 310 nm, and 320 nm.

the lag at lower doses was longer compared to 297 nm. No tailing was reached at 310 nm. At 320 nm the *E. coli* inactivation was minimal because the time required to reach higher doses was getting prohibitively long. For similar reasons, we did not see any measurable inactivation at wavelengths higher than 320 nm. The action spectra for bacteria *E. coli* (ATCC 15597) was complemented with data for other strains of *E. coli* (*E. coli* O157 and *E. coli I5 t-u-a), Vibrio cholerae*, and *Salmonella* (Figure 7). Similar to viruses, the inactivation constant of *E. coli* reduces (at more or less second-order polynomial trend) as wavelength increases but there is a steep drop in inactivation above 280 nm.

Photoreactivation

After *E. coli* (ATCC 15597) was exposed to a 297 nm radiation, the inactivation dose of 219 mJ/cm^2 left approximately 1.25 $\log_{10}$ CFU/mL in suspension, and within 1 hour of exposure to light from an incandescent lamp, about 0.5 $\log_{10}$ CFU/mL of bacteria had recovered (Figure 8). The increase due to repair was almost linear with time over the 3-hour period of exposure to radiation from an incandescent lamp. The second suspension with no detectable *E. coli* (0 CFU/mL) after inactivation with a higher dose of 314 mJ/cm^2 yielded reactivation of approximately 0.2 $\log_{10}$ units within 2 hours and 0.5 $\log_{10}$ units after 4 hours. Further, the repair trend started flattening out with approximately 0.6 $\log_{10}$ units increase after 6 hours of exposure (Figure 8). A third bacterial suspension

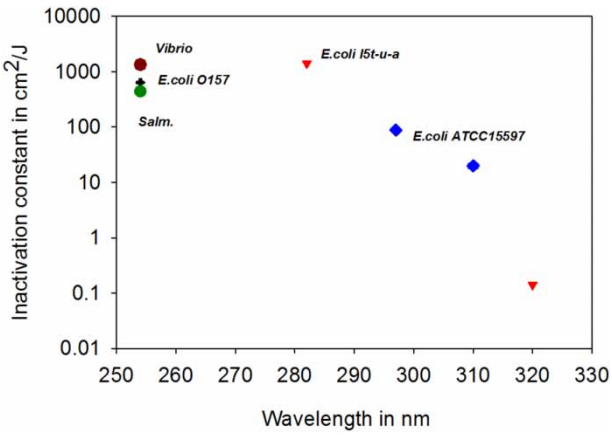

Figure 7 | Bacterial action spectra: ♦ *E. coli* ATCC15597, ● *Vibrio*, ■ *Salmonella*, ♠ *E. coli* O157 from Hijnen & Medema (2005); ▼ *E. coli I5t-u-a* Boyce & Setlow (1963).

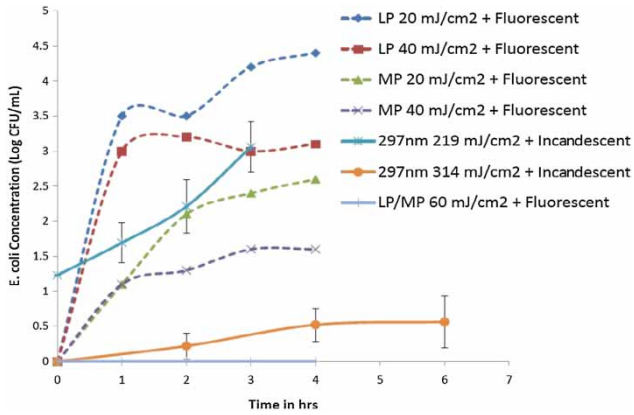

Figure 8 | *E. coli* ATCC 15597 photorepair compared with repair caused by LP and MP lamps (LP and MP data were extracted from Quek & Hu (2008a)). *Note:* Error bars show standard deviation around the mean.

was incubated in the dark; no evidence of (dark) repair was observed.

For comparison, the results of similar experiments involving LP and MP (UVC) sources are included in Figure 8; these data were extracted from the work of Quek & Hu (2008a) and Zimmer & Slawson (2002). When exposed to radiation from LP and MP sources with inactivation doses less than 60 mJ/cm^2, photorepair of *E. coli* was observed. When *E. coli* ATCC 15597 was subjected to 20 mJ/cm^2 using a LP lamp, no viable bacteria were detected; however, after 1 hour of exposure to output from a fluorescent lamp, 3.5 $\log_{10}$ units of recovery were observed. Continued exposure to output from the fluorescent lamp led to flattening of the recovery process at a plateau of approximately 4.4 $\log_{10}$ units. Similar behavior was observed for 40 mJ/cm^2 dose delivered using a LP lamp.

DISCUSSION

Wavelengths in the UVB and UVA ranges can be used to inactivate both bacteria and viruses; however, the doses required to accomplish a given level of inactivation are considerably larger than those commonly applied with UVC radiation. The inactivation behavior in the UVB range appears to follow a trend that mimics the absorbance spectra of nucleic acids, which suggests that the mechanism of inactivation is similar to that of UVC radiation. These trends were evident with MS2, T4, and *E. coli*. This agrees with Jagger (1981) who reported that UV inactivation in UVB (290–320 nm) and UVC (below 290 nm) result from a similar path of dimerization of some DNA bases, whereas, the exposure to UVA (320–400 nm) causes sublethal effects such as growth delay, denaturing of proteins, and impaired membrane transport for bacteria.

Most investigations of UVB- and UVA-based disinfection performed to date have involved polychromatic UV sources, such as solar simulators or ambient solar radiation (Heaselgrave *et al.* 2006; McGuigan *et al.* 2006; Ubomba-Jaswa *et al.* 2009). Studies of this nature provide critical information regarding the practical application of solar UV-based processes; however, they fail to define the wavelength dependence of these disinfection processes. Knowledge of the wavelength-dependent behaviors (i.e., action spectra) can inform the design of improved solar UV disinfection systems by identifying wavelength ranges that contribute to overall microbial inactivation. By extension, this information can be used in the identification and selection of materials of construction that have optical properties to maximize availability and application of radiation from the portions of the solar spectrum that contribute most effectively to microbial inactivation, as shown by Mbonimpa *et al.* (2012).

The design of SODIS systems should also account for deviations from commonly assumed single-event (i.e., first-order) dose-response behavior. As indicated in this study, bacteria may display deviations from single-event behaviors, including a lag and tailing. These behaviors have also been reported for systems based on UVC radiation. Dose-response models, such as the Phenotypic Persistence and External Shielding model have been demonstrated to be effective for describing these common deviations (Pennell *et al.* 2008).

Bacterial repair observed in this study is also a concern for disinfection systems based on UVB radiation. Evidence of photorepair was presented that was qualitatively similar to behavior that had been previously reported for systems based on UVC radiation. Photorepair of *E. coli* following UVC exposure has been widely reported in the literature. For instance, *E. coli* has been reported to include 20 photolyase enzymes, each with the ability to repair nominally five dimers per minute (Zimmer & Slawson 2002). Given that

the mechanism of inactivation for UVB radiation appears to be similar to that of UVC radiation, it is perhaps not surprising that similar repair behavior would be observed as well. Jagger & Stafford (1965) also indicated that repair depends on inactivation wavelength, type of microorganism, and the growth phase during the inactivation process. They indicated that some microorganisms can exhibit first-order reactivation and other complex behaviors. This variability could be linked to differences in molecular components, such as whether a reactivable site is in DNA or RNA, or whether the site may be in the nucleus or cytoplasm (Jagger 1958). This implies an additional dose above the dose for inactivation may be required for SODIS systems to achieve un-repairable damage for bacteria.

In this study, experiments were conducted using non-pathogenic surrogates; great care must be used in translating these bench-scale results into full-scale disinfection systems. However, there are numerous precedents using surrogates in the disinfection of water. For instance, MS2 and T4 are commonly applied as surrogates for viral microorganisms in water disinfection (Mamane-Gravetz *et al.* 2005).

The work described herein has demonstrated that inactivation responses of *E. coli* 15597 were similar to those of several pathogenic bacteria, including *Vibrio cholerae*, *Salmonella*, and *E. coli* O157. The summary of Wright & Cairns (1998) indicated that bacteria generally have an inactivation peak around 260 nm, which reduces as wavelength increases and that this behavior is linked to DNA absorption of UV.

For repair tests, *E. coli* (ATCC 15597) may also represent an appropriate surrogate since it is known to have repair mechanisms that are more active than many bacterial species of concern, including *E. coli* O157 (Quek & Hu 2008a). No evidence of dark repair was observed with *E. coli* (ATCC 15597), a result that is in agreement with Oguma *et al.* (2001).

This study demonstrated wavelength-dependent behaviors that find application in solar disinfection studies (SODIS). The inactivation profiles for double-stranded DNA bacteriophage MS2 and single-stranded RNA bacteriophage were log-linear, and for both phages, inactivation decreased (linear trend) with an increase in wavelength. The dose-response behavior of bacteria was also log-linear, but with noticeable lag (at 297 nm and 310 nm) and tailing phases (at 297 nm).

The results of this work establish the dose ranges that are required for successful inactivation, as well as the conditions that may trigger regrowth due to photorepair mechanisms. These findings help fill data gaps in the literature concerning SODIS.

It is also important to note that the performance of SODIS systems will be influenced by materials' specifications. For example, materials must be selected that enhance the collection of more germicidally active solar radiation (UVB and lower end of UVA). Previous studies recommended PET bottles for SODIS (McGuigan *et al.* 1998; Wegelin *et al.* 2001), despite the fact that PET is essentially opaque to UVB radiation (Mbonimpa *et al.* 2012). Others have explored potential SODIS materials with a transmittance in UVB; these include polyethylene (PE) and low-density polyethylene (Lawrie *et al.* 2015; Danwittayakul *et al.* 2017).

CONCLUSIONS

Laboratory experiments with MS2 and T4 bacteriophages and *E. coli* demonstrated the effectiveness of solar UV radiation for inactivating viruses and bacteria. The germicidal effect was only observed in wavelengths below 320 nm of the electromagnetic spectrum. The capacity of bacteria to repair the damage was observed for *E. coli*. These results are useful for designing solar disinfection systems. However, we do recommend further studies using other microorganisms such as protozoa and spores.

DISCLAIMER

The views expressed in this paper are those of the authors and do not reflect the official policy or position of the US Air Force, the DoD, or the US Government.

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