# MS2 coliphage and *E. coli* UVB inactivation rates in optically clear water: dose, dose rate and temperature dependence

Yu Lian, Lei Mai, Nancy Cromar, Neil Buchanan, Howard Fallowfield and Xiaoming Li

## ABSTRACT

Natural ultraviolet irradiance disinfection is known to play a significant role in both natural wastewater treatment systems and drinking water disinfection processes, while the influence of ultraviolet B (UVB) delivering method on sunlight disinfection outcome is still unclear. This study aims to determine the effects of environmentally relevant temperatures, UVB doses ($J\,m^{-2}$) and dose rates ($W\,m^{-2}$) on the inactivation and log reduction values (LRVs) of the F-RNA coliphage MS2 and *Escherichia coli* in optically clear water. *E. coli* and MS2 were separately incubated and irradiated at five different doses of UVB light that delivered using six UVB dose rates. The results of the study demonstrate that the UVB dose delivering method (combination of dose rate and exposure time) influences inactivation and LRVs of *E. coli* and MS2 at all UVB doses investigated (up to seven-fold difference). Two phases were identified within the UVB dose rate, UVB inactivation or LRV curves for both organisms; a UVB dose rate limited inactivation phase and a dose rate saturation inactivation phase. The results contribute to a better understanding of UVB disinfection in the environment and natural wastewater treatment systems, potentially improving the design and operation of high rate algal ponds.

**Key words** | dose, dose rate, *E. coli*, inactivation, MS2, temperature, UVB

**Yu Lian** (corresponding author)
**Xiaoming Li**
Key Laboratory of Environmental Biology and
  Pollution Control, School of Environmental
  Science and Engineering,
Hunan University,
Changsha, Hunan Province 410082,
China
E-mail: *lian0100@hnu.edu.cn*

**Yu Lian**
**Nancy Cromar**
**Neil Buchanan**
**Howard Fallowfield**
Health and the Environment Group; School of the
  Environment,
Flinders University,
Adelaide, South Australia 5042,
Australia

**Lei Mai**
Key Laboratory of Environmental Pollution and
  Health, School of Environment,
Jinan University,
Guangzhou 510632,
China

## ABBREVIATIONS

| | |
|---|---|
| *E. coli* | *Escherichia coli* |
| HRAP | High-rate algal pond |
| $K_i$ | Inactivation rate |
| LRV | $Log_{10}$ reduction value |
| UV | Ultraviolet |
| UVB | Ultraviolet B |
| UVC | Ultraviolet C |
| WSP | Wastewater stabilization pond |

## INTRODUCTION

Sunlight provides a cost-effective, highly accessible and sustainable natural disinfection method. These unique advantages have attracted considerable research activities seeking to understand the mechanism and take better advantage of the resource. Sunlight is prevalently used for drinking water purification (Zyara *et al.* 2016). This also includes solar water disinfection (SODIS) (Hashimoto *et al.* 2005; Fisher *et al.* 2012; Mbonimpa *et al.* 2012) and 'natural' wastewater treatment systems including wetlands, waste stabilization ponds (WSPs) and high rate algal ponds (HRAPs) (Craggs *et al.* 2004; Diamond *et al.* 2005; Bolton *et al.* 2010, 2011). These natural wastewater treatment systems offer the advantage of reduced energy consumption and are suitable for rural and peri-urban communities where the treated wastewater can be beneficially reused (Li *et al.* 2018). It is important that the risk of exposure of the public to pathogens potentially present in reused water is adequately managed (Young *et al.* 2016). The design and operation of natural wastewater treatment systems, to improve disinfection efficiency, would be aided by a better understanding of the mechanism contributing to

inactivation of faecal indicator microorganisms such as *Escherichia coli* and the F-specific RNA coliphage MS2.

Solar disinfection is attributed to the effect of ultraviolet radiation which is divided into three wavelength dependent regions of ultraviolet light (UV). UVA (320–400 nm) is the predominant wavelength reaching the Earth's surface followed by UVB (280–320) and UVC (200–280 nm), the latter is all absorbed by the Earth's atmosphere. UVB causes direct photoinactivation via damage to DNA, RNA and other cell constituents; in the meantime, it is recognized as the primary germicidal component in sunlight that reaches the Earth's surface, and therefore contributes a lot to the natural wastewater treatment system disinfection. The short wavelength UVB, however, is rapidly attenuated with depth by suspended solids and dissolved organic matter present within wastewater in WSPs and HRAPs. Bolton (2011) found that 99% of the incident UVB was attenuated at a depth of $0.08 \pm 0.03$ m in an unmixed, 1.5 m-deep WSP, suggesting only about 5% of the pond depth was exposed to UVB irradiation. Nevertheless, the HRAPs were operated at depth between 0.2 m and 0.5 m, so as to 16% of the pond depth being exposed to UVB irradiation. The intentional paddlewheel mixing of HRAP also ensures increased exposure through the UVB photic zone, which undoubtedly has variable UVB light doses and dose rates for both depth and attenuation. UV disinfection can sufficiently decrease the *E. coli* culturability (Zhang *et al.* 2015). There is a need to better understand the role of UVB dose and dose rate on the inactivation of faecal indicator organisms such as MS2 and *E. coli* in such optically complex aquatic environments.

The Bunsen & Roscoe (1857) reciprocity law states that a photo-biological effect is proportional to the total energy dose and is independent of how that dose is delivered, that is, the combination of intensity and exposure time. The outcome of UV irradiation in biological systems, however, also depends on other interactions, for example, the presence of endogenous and exogenous photosensitizers which may modulate the response (Vermeulen *et al.* 2008); the MS2 inactivation had been found to not be influenced by endogenous photo-oxidation due to its lack of chromophores (Kohn & Nelson 2006). However, it is recognized that the influence of how the total energy dose is delivered, the exposure–intensity relationship, on the biological effect – such as inactivation – needs to be determined in individual cases. The dose rate and exposure time required to deliver a specific UV dose may also influence the disinfection outcome, however, the relevant studies have mainly considered the UVC disinfection process for production of

drinking water (Murakami *et al.* 2006). Many authors report the UV dose as the sole descriptive parameter (Hijnen *et al.* 2006). This appears to ignore the influence of how that UV dose was delivered. Furthermore, the influence of ambient temperature is also frequently ignored.

UV irradiances have been shown to interact with many environmental factors such as the temperature, pH and dissolved oxygen (DO) (Kadir & Nelson 2014): among them, temperature was recognized as one of the most important factors (Theitler *et al.* 2012). There is a paucity of information regarding the interaction between UVB and temperature on the outcome of inactivation of *E. coli* and MS2 in different types of wastewater. The research reported here is an initial study to determine the effect of UVB dose, dose rate and temperature on the inactivation of F-RNA coliphage MS2 and *E. coli*, which is important and rarely been studied. This study was conducted in optically clear water rather than wastewater to minimize confounding effects of attenuation and to determine potentially maximum UVB inactivation rates and LRVs for these organisms.

The objectives of this study were to (1) determine the influence of environmentally relevant UVB dose ($Jm^{-2}$), dose rate ($Wm^{-2}$) and exposure time and incubation temperature on the inactivation of *E. coli* and MS2 in optically clear water and (2) create a unique database for modeling disinfection effect of UVB irradiance. These contribute to a broader objective to better understand natural UVB disinfection and to improve the design, operation and management of natural wastewater treatment systems, especially HRAPs.

## MATERIALS AND METHODS

This study explored the effect of three environmentally relevant temperatures on the MS2 inactivation rate in pH 7.5 buffered RO water under a range of UVB dose rates ($Wm^{-2}$). The exposure times at specific UVB dose rates were calculated to maintain a constant dose ($Jm^{-2}$), both at each sampling time and at the end of the incubation for each dose rate.

### Stock preparation and quantification of MS2 and *E. coli*

The MS2 stock (ATCC #15597-B1) was prepared by flooding a plate of host *E. coli* containing over 100 MS2 plaques with 5 mL half-strength tryptone water (Oxoid™). The plate was incubated for 30 min at 37 °C with swirling carried out every 10 min; the suspension was then decanted and filtered

(0.2 μm) to remove the host *E. coli*. The final MS2 stock solution was stored at 4 °C in 100 mL half-strength tryptone water with 10% sterile glycerol, and 1 mL of suspension was inoculated into the 300 mL pH 7.0 buffered reverse osmosis (RO) water before running the experiments. In addition, The *E. coli* stock (ATCC #19434) was inoculated into 10 mL sterile nutrient broth (Oxoid™) and grown at 37 °C overnight. The *E. coli* stock was used immediately after incubation by inoculating 1 mL of suspension into the 300 mL pH 7.0 buffered RO water.

Quantification of MS2 was accomplished by the double layer method (Noble *et al.* 2004; Young *et al.* 2016), when required, samples were serially diluted 1 mL into 9 mL sterile 0.5% tryptone water. Plates were incubated at 35 °C for 24 h and the MS2 count expressed as plaque-forming units (PFUs) 100 ml$^{-1}$. *E. coli* was enumerated using the defined substrate Colilert™ method (IDEXX Laboratories, Maine, USA), as per the manufacturers' instructions. Where necessary, the *E. coli* were serially diluted 1 mL in 9 mL sterile 0.1% peptone water (Oxoid™). The Quanti-tray®/2000 was incubated at 35 ± 0.5 °C for 24 h; the *E. coli* count was expressed as the most probable number (MPN) 100 mL$^{-1}$.

*E. coli* LRVs were determined at five UVB doses (J m$^{-2}$), which were achieved using five UVB dose rate (W m$^{-2}$) – exposure time combinations. Samples for enumeration were taken in accordance with the study design's time points (Table 1) to ensure consistent exposure to the selected UVB doses. Two-way ANOVA and follow-up least significant difference (LSD) analysis were applied to compare the significance of UVB dose and dose rates on *E. coli* log reduction value (LRV).

**Table 1** | Sampling timetable using six ordinal, environmentally relevant UVB dose rates (Wm$^{-2}$) to achieve five UVB doses (Jm$^{-2}$) to determine

| Dose rate (Wm$^{-2}$) | Elapsed time at each sampling point (h) | | | | |
| --- | --- | --- | --- | --- | --- |
| | 1st | 2nd | 3rd | 4th | 5th |
| 0.5 | 3.4 | 12.25 | 21.10 | 34.57 | 48.05 |
| 1 | 1.7 | 6.12 | 10.55 | 17.29 | 24.02 |
| 2 | 0.85 | 3.06 | 5.27 | 8.64 | 12.01 |
| 3 | 0.57 | 2.04 | 3.52 | 5.76 | 8.01 |
| 4.5 | 0.38 | 1.36 | 2.34 | 3.84 | 5.34 |
| 6.0 | 0.28 | 1.02 | 1.76 | 2.88 | 4.00 |
| **Dose received (Jm$^{-2}$)** | 6,126 | 22,049 | 37,973 | 62,232 | 86,490 |

*Note:* UVB inactivation of *E. coli* shared the same sampling timetable except for the 6.0 Wm$^{-2}$ dose rate, which was excluded from the study.

The detailed methods for the preparation of buffered matrix and UVB irradiation can be found in the supplementary information (available with the online version of this paper).

## Experimental design

Bolton (2011) collected environmental UVB data over 28 randomly assigned days in 2009 using a spectroradiometer (PMA2106-WP; Solar Light) placed on the roof of Flinders Medical Centre (35°01′S and 38°34′E) as a typical database, Adelaide, South Australia. The UVB dose ranged from 6,126 Jm$^{-2}$ to 86,490 Jm$^{-2}$. The average daily UVB dose was 37,673 Jm$^{-2}$. The dose rates ranged from 0 Wm$^{-2}$ to 4.04 Wm$^{-2}$. Six ordinal UVB dose rates of 0.5, 1.0, 2.0, 3.0, 4.5 and 6.0 Wm$^{-2}$, and five UVB doses of 6,126, 22,049, 39,973, 62,232 and 86,490 Jm$^{-2}$ were chosen for the MS2 inactivation experiments. The same UVB doses and dose rates were applied for determination of *E. coli* inactivation, except that the 6 Wm$^{-2}$ UVB dose rate was omitted. The duration of incubation to achieve the UVB dose (Jm$^{-2}$) at various dose rates (Wm$^{-2}$) is shown in Table 1. Dark incubated controls (incubation in dark all the time) for both MS2 and *E. coli* at the respective incubation temperatures were also included in the study.

## Statistical analysis

The Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFiT) was used to calculate the maximum UVB inactivation rate, $K_i$ (Geeraerd *et al.* 2005). GinaFit is a freeware tool to assess non-log-linear microbial survivor curves, 10 different types of microbial survival models, and the 10 different models involved are: log-linear regression; log-linear + shoulder; log-linear + tail; log-linear + shoulder + tail; Weibull; Weibull with fixed parameter p; Weibull + tail; double Weibull; biphasic model; and biphasic and shoulder. Initially, each of the 10 models available within GinaFiT were applied to each experimentally derived inactivation curve, to identify the model reporting the smallest root mean sum of squared errors (RSME). Generally, MS2 UVB inactivation was best described using the log$_{10}$-linear + tail model, whereas the log$_{10}$-linear model was the best fit for MS2 dark control groups, as they demonstrated neither a shoulder nor a tail in the inactivation plots. GInaFiT has also been successfully used to fit *E. coli* solar disinfection (SODIS) inactivation data (Berney *et al.* 2006).

The log$_{10}$ reduction value (LRV) was used to analyse the relationship between UVB dose (Jm$^{-2}$) and dose rate (Wm$^{-2}$). The LRV was calculated at each sampling time to

describe the reduction in numbers of either *E. coli* or MS2 with incubation time as shown in Equation (1).

$$LRV = \log_{10} \frac{N_t}{N_0} \qquad (1)$$

where $N_t$ is the number of pathogen indicators at time t; and $N_0$ is the number of pathogen indicators at time 0. Two-way analysis of variance (ANOVA; SPSS) was applied to the data to determine the significance of dose, dose rate and temperature on MS2 and *E. coli* inactivation rates.

## RESULTS AND DISCUSSION

### Temperature effects on MS2 UVB inactivation

Dark inactivation of MS2 over the 48-h incubation period was minimal at all three incubation temperatures (Table 2). There was no statistically significant difference (two-way ANOVA) in MS2 inactivation rate ($K_i$) at 10 °C, 20 °C and 30 °C in the dark control incubations.

**Table 2** │ MS2 inactivation rates $K_i$ ($h^{-1}$) in buffered pH 7.5 RO water at UVB dose rates of 0.5, 1, 2, 3, 4.5 and 6 $Wm^{-2}$ (at a constant dose of 86.49 kJ $m^{-2}$) at 10 °C, 20 °C and 30 °C

| Temperature (°C) | UVB dose rate ($Wm^{-2}$) | Incubation time (h) | $K_i$ ($h^{-1}$) | SD | $R^2$ | n |
|---|---|---|---|---|---|---|
| 10 | Dark Control | 48 | 0.01 | 0.01 | 0.4146 | 18 |
|  | 0.5 | 48.05 | 0.06 | 0.03 | 0.9056 | 18 |
|  | 1 | 24.02 | 0.34 | 0.02 | 0.9971 | 18 |
|  | 2 | 12.01 | 1.31 | 0.13 | 0.9853 | 18 |
|  | 4 | 8.01 | 2.17 | 0.03 | 0.9998 | 18 |
|  | 4.5 | 5.34 | 2.87 | 0.13 | 0.9973 | 18 |
|  | 6 | 4.00 | 2.68 | 0.15 | 0.996 | 18 |
| 20 | Dark Control | 48 | 0.02 | 0.00 | 0.9725 | 18 |
|  | 0.5 | 48.05 | 0.08 | 0 | 0.9976 | 18 |
|  | 1 | 24.02 | 0.35 | 0.01 | 0.9994 | 18 |
|  | 2 | 12.01 | 1.27 | 0.04 | 0.9982 | 18 |
|  | 3 | 8.01 | 2.51 | 0.05 | 0.9993 | 18 |
|  | 4.5 | 5.34 | 2.84 | 0.09 | 0.9988 | 18 |
|  | 6 | 4.00 | 3.02 | 0.14 | 0.9971 | 18 |
| 30 | Dark Control | 48 | 0.00 | 0.01 | 0.01 | 18 |
|  | 0.5 | 48.05 | 0.16 | 0.01 | 0.9978 | 18 |
|  | 1 | 24.02 | 0.96 | 0.1 | 0.9894 | 18 |
|  | 2 | 12.01 | 1.82 | 0.13 | 0.9952 | 18 |
|  | 3 | 8.01 | 2.63 | 0.07 | 0.9987 | 18 |
|  | 4.5 | 5.34 | 3.4 | 0.11 | 0.9983 | 18 |
|  | 6 | 4.00 | 3.71 | 0.11 | 0.9988 | 18 |

*Notes: $K_i$ ($h^{-1}$) (mean ± SD) was obtained from a $\log_{10}$-linear + tail model using the GInaFiT model (Geeraerd et al. 2005) for which the individual $R^2$ values are shown together with n, the number of data points included in the model.*

The MS2 UVB inactivation rate constants ($K_i$) for UVB dose rates ranging from 0.5–6.0 $Wm^{-2}$ at 10 °C, 20 °C and 30 °C are shown in Table 2. The highest $K_i$ was 3.71 $h^{-1}$ at 30 °C, 6 $Wm^{-2}$, while the lowest $K_i$ was 0.06 $h^{-1}$ at 10 °C, 0.5 $Wm^{-2}$. The MS2 inactivation rates $K_i$ at 30 °C were significantly higher than those recorded at 10 °C ($p < 0.001$). A similar statistically significant difference was also observed between $K_i$ at 20 °C and 30 °C ($p < 0.001$). However, no statistically significant difference in $K_i$ was identified between 10 °C and 20 °C ($p = 0.179$).

Preliminary analysis showed that a linear approximation gave a poor relationship between the measured and modeled values. Consequently, growth curve analysis (Mirman 2014) was used to model the data on the effect of temperature on $K_i$ on UVB-induced inactivation of MS2 at dose rates from 0.5 $Wm^{-2}$ to 6 $Wm^{-2}$. The interaction between $K_i$ and dose rate was modeled using a third-order (cubic) orthogonal polynomial for water temperature 10 °C, 20 °C and 30 °C (Figure 1; Equations (2)–(4) in the supplementary information, available with the online version of this paper).

Generally, at all incubation temperatures, the MS2 inactivation rates $K_i$ increased significantly ($p < 0.001$) as the UVB dose rates increased from 0.5 $Wm^{-2}$ to 6 $Wm^{-2}$. However, the only exceptional $K_i$ appeared between dose rates of 4.5 $Wm^{-2}$ to 6 $Wm^{-2}$ at 10 °C and 20 °C. The dose rate of 6 $Wm^{-2}$ was higher than reported environmental UVB dose rates. The data suggest that the effect of UVB dose rate on $K_i$ saturates at around 6 $Wm^{-2}$.

MS2 UVB inactivation rates ($K_i$) were influenced by temperature with significantly greater inactivation occurring at 30 °C than either at 10 °C or 20 °C in the buffered (pH 7.5) RO water. A few studies considered the effect of temperature on UV disinfection: Theitler *et al.* (2012) concluded there was a slight synergistic effect between heat and natural sunlight on MS2 inactivation in deionized water, however, the incubation temperature was as high as 59 °C. The absorbance of UVB by RNA to form pyrimidine dimers causes the direct photo-inactivation of MS2. Higher temperatures accelerate the formation of the double bond between pyrimidine molecules, increasing the rate of inactivation of MS2 (USEPA 1996). As well, higher temperatures could also affect the correct folding of the viral coat protein and then increase the inactivation synergistically (Torres *et al.* 2016).

The *E. coli* UVB inactivation rate was determined at 20 °C and 30 °C. In contrast to the MS2 incubations, no significant temperature effect on *E. coli* inactivation rates were observed over a range of UVB dose rates between 20 °C and 30 °C. Wegelin *et al.* (1994), using a rotating photoreactor to explore the influence of temperature on the process of solar
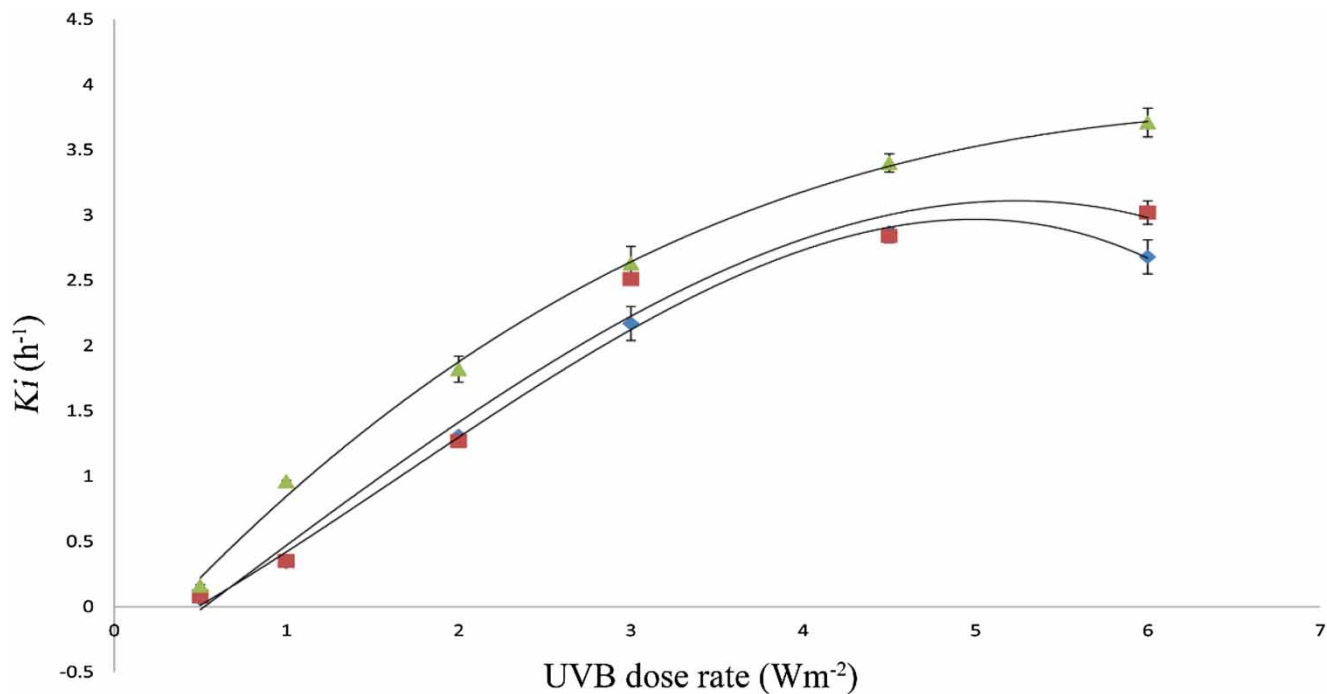
**Figure 1** │ Measured MS2 inactivation rate $K_i$ ($h^{-1}$) in buffered pH 7.5 RO water at 0.5, 1, 2, 3, 4.5 and 6 $Wm^{-2}$ for 10 °C (♦), 20 °C (■) and 30 °C (▲); experimental data 'fitted' using a cubic polynomial mode.

water disinfection (SODIS), reported that *E. coli* UV survival behaviour did not change from 20 °C to 40 °C; however, at 50 °C the inactivation rate was almost three times higher than that recorded between 20 °C and 40 °C. *Streptococcus faecalis* was also included in their study (Wegelin *et al.* 1994) and its response was similar to *E. coli*; however, *Enterococcus* responded differently, with the inactivation rate only increasing above 55 °C. Theitler *et al.* (2012) also recorded up to three-log difference in *E. coli* inactivation difference for the synergistic effect of 50 °C and 2,000 $KJm^{-2}$ under full wavelength natural sunlight or simulated solar irradiance when compared to the temperature-irradiance separate treatment. The difference in the effect of temperature between *E. coli* and MS2 may be explained by possession of two associated DNA repair mechanisms by *E. coli* (Chan & Killick 1995), which also influences the outcome of inactivation under UVB irradiance. Firstly, after the formation of lethal pyrimidine dimers, a photoreactivation light and a photo-reactivating enzyme contribute to separating the dimers. Secondly, a dark repair system removes the dimers. The enzyme systems involved in repairing DNA damage have been shown to be temperature-dependent; thus, at higher temperatures, the repair system works more efficiently, including within the temperature range from 20 °C to 30 °C used in the study reported here.

## Interaction between UVB dose rate ($Wm^{-2}$) and UVB dose ($Jm^{-2}$) on MS2 UVB inactivation

The effect of UVB dose delivery on MS2 inactivation was explored in buffered (pH 7.5) RO water at 10 °C, 20 °C and 30 °C. Samples were taken in accordance with the study design's time points (Table 1) to ensure that the UVB dose ($Jm^{-2}$) was internally consistent at each sampling time in the sequence. Two-way ANOVA and follow-up LSD analysis were applied to compare the significance of UVB dose and dose rates on MS2 $\log_{10}$ reduction value (LRV).

The MS2 LRV increased with increasing UVB dose ($Jm^{-2}$) at all incubation temperatures (Figure 2(a)–2(c)). At a dose equivalent to the daily UVB dose (6,126 $Jm^{-2}$) recorded for a cloudy winter's day in South Australia, the MS2 LRV was <1.0 at all incubation temperatures. In contrast, at a UVB dose of 89,490 $Jm^{-2}$, which was above the maximum UVB dose (86,490 $Jm^{-2}$) recorded on a sunny summer's day in South Australia, the MS2 LRV was up to seven-fold higher.

Notwithstanding, it is clear from Figure 2(a)–2(c) that the manner in which the UVB dose ($Wm^{-2}$) was delivered, that is, the dose rate ($Wm^{-2}$)–exposure time relationship employed to achieve the dose ($Jm^{-2}$), influenced the LRV at all doses and at all temperatures except for the lowest
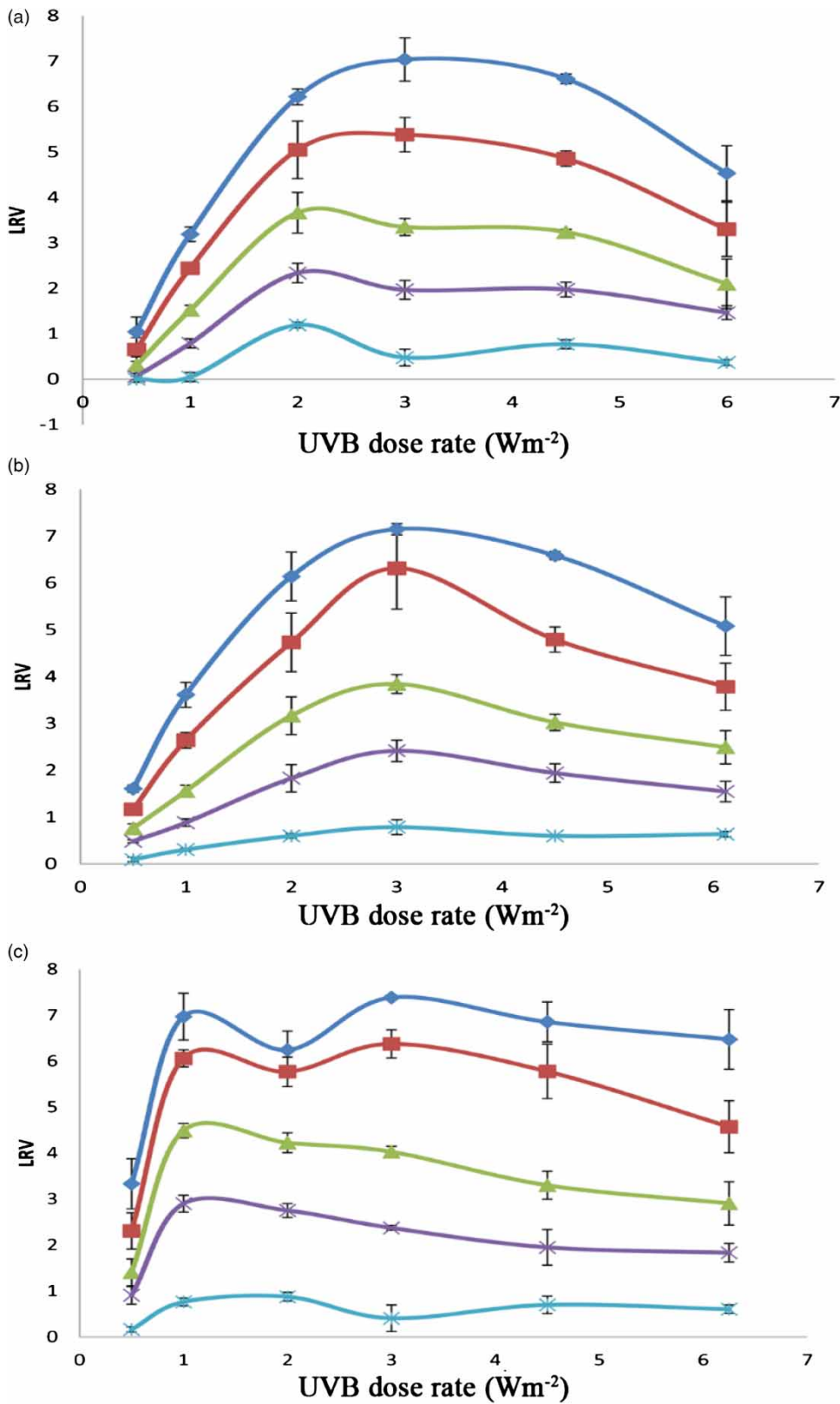
**Figure 2** │ MS2 $\log_{10}$ reduction value (LRV) in buffered pH 7.5 RO water, using six UVB dose rates–time combinations: 0.5, 1, 2, 3, 4.5 and 6 $Wm^{-2}$, to achieve five UVB doses: 6,126($\ast$), 22,049($\times$), 37,973(▲), 62,231(■) and 86,490(◆) $Jm^{-2}$ at (a) 10 °C, (b) 20 °C and (c) 30 °C.

dose, 6,126 Jm$^{-2}$ at 20 °C and 30 °C. The MS2 LRVs for all UVB doses in incubations at 10 °C and 20 °C increased sharply with increasing dose rates from 0.5 Wm$^{-2}$ to 2.0 Wm$^{-2}$, which could be defined as a 'dose rate-limited' stage: in the 'dose rate-limited stage', the increase in dose rate increased the LRV. The LRV plateaued at a dose rate between 2.0 Wm$^{-2}$ and 6.0 Wm$^{-2}$, suggesting a 'dose rate-saturated' stage, where an increase in UVB dose rate has little influence on LRV. Dissimilarly, at 30 °C, the dose rate-limited stage appeared shorter than 10 °C and 20 °C, from 0.5 Wm$^{-2}$ to 1.0 Wm$^{-2}$ for all UVB doses (Jm$^{-2}$); above 1 Wm$^{-2}$, all curves at 30 °C appeared 'dose rate-saturated'. This increase of LRV by increasing dose rate and adjusting incubation time to yield the same dose was more evident at doses >6,126 Jm$^{-2}$ at all incubation temperatures. Interestingly, the LRV at all doses (Jm$^{-2}$) decreased as the dose rate–time combinations approached the environmental recorded maximum dose rate of 4.5 Wm$^{-2}$. UVB dose rate had a statistically significant influence on MS2 LRV under the same UVB doses at all three temperatures ($p < 0.001$); also, significant interaction was found between the effect of UVB doses and UVB dose rates on MS2 LRV.

As mentioned above, the UVB dose rates significantly influenced the LRV at the same UVB dose ($p < 0.001$) at 10 °C: the LRV increased from 0.5 Wm$^{-2}$ to 2 Wm$^{-2}$ and then decreased above 4.5 Wm$^{-2}$. The highest LRV achieved at each dose was between 2 Wm$^{-2}$ and 3 Wm$^{-2}$ while the lowest LRV was at 0.5 Wm$^{-2}$. As shown in Figure 2(b), at 20 °C, although delivering UVB doses by different UVB dose rates significantly influences the LRV ($p < 0.001$) except at the lowest UVB dose of 6,126 Jm$^{-2}$, where the effect of five UVB dose rates on the recorded LRV was statistically insignificant ($p = 0.093 > 0.05$). This finding confirmed the conclusion that the UVB dose rate effect was less apparent at lower UVB doses. Figure 2(c) shows the UVB dose rate effect at 30 °C in RO water. As at 20 °C, the UVB dose rate imposed significantly influenced ($p < 0.05$) LRV at all UVB doses except for the lowest one, 6,126 Jm$^{-2}$ ($p = 0.13$).

Overall, at almost all UVB doses, the imposed UVB dose rate significantly influenced the MS2 LRV at all three temperatures. The two stages, 'dose rate-limited' and 'dose rate-saturated', were observed at all three temperatures. Commonly, at the high UVB dose, the effect of UVB dose rate was more apparent than at low UVB doses. For all curves, the lowest LRV achieved were at 0.5 Wm$^{-2}$, and the highest occurred in the UVB dose rate range (1–3 Wm$^{-2}$), which was within the observed environmental range of UVB dose rates.

Six environmentally relevant UVB dose rates (Wm$^{-2}$) were selected to assess the influence of dose rate on MS2 inactivation rates in RO water. Considering that direct photo-inactivation is the mechanism of UVB inactivation, it was predicted that the MS2 inactivation rate ($K_i$) would increase with increasing UVB dose rate as a consequence of the increased rate of formation of RNA pyrimidine dimers. The results confirmed this hypothesis, the inactivation rate of MS2 increased with increases in UVB dose rate (Wm$^{-2}$). Further, the results suggested two phases of inactivation of MS2 in response to UVB; a UVB dose rate limited phase from 0.5 to 4.5 Wm$^{-2}$ and a dose rate saturated phase at UVB dose rates >4.5 W m$^{-2}$.

Few studies have explored the effect of UVB dose rate on waterborne pathogen indicators. Since none were provided, first order MS2 inactivation rates were calculated from the data of Sommer *et al.* (1998). These demonstrated that while maintaining the same UVC dose (100 or 300 Jm$^{-2}$) the MS2 inactivation rate increased with increases in the UVC dose rate (0.02–2.0 Wm$^{-2}$). This is consistent with the results of the UVB study presented here. There was no evidence of a UVC dose rate saturated phase of inactivation, which was likely due to the low UVC dose rates employed not enabling saturation to be reached. Liu & Zhang (2006) assessed the effect of UVC dose rate and turbidity on pathogen indicator inactivation. In their experiments, low pressure mercury vapor lamps (253.7 nm) provided three UVC dose rates, 0.08 Wm$^{-2}$, 1 Wm$^{-2}$ and 1.8 Wm$^{-2}$, and kaolin were added to sterilized water to obtain three levels of turbidity (0.5, 4 and 12 NTU). First order inactivation rates were again calculated from the MS2 log$_{10}$ reduction value and exposure time data provided by Liu & Zhang (2006). This showed again that when UVC dose rate increased, the MS2 inactivation rate also increased – UVC dose rate limited inactivation. There was a similar absence of a UVC dose rate saturated phase of inactivation.

Although the data were fewer for *E. coli* due to its greater sensitivity to UVB, it was clear that as for MS2, inactivation increased with increasing UVB dose rates between 0.02 and 2.0 Wm$^{-2}$ at 20 °C and 30 °C. Derivation of inactivation rates for three different strains of *E. coli*, exposed to UVC by Sommer *et al.* (1998) also showed that the *E. coli* inactivation rate increased with increasing UVC dose rate; a finding also supported by the results of Liu & Zhang (2006).

The Bunsen & Roscoe (1857) reciprocity law states that a photo-biological effect is proportional to the total energy dose and is independent of how that dose is delivered, however, the outcome may also depend on other interactions and therefore requires characterization for individual systems. Furthermore, in most studies of UV disinfection, only the UV dose was recorded to describe UV irradiance

(Chang 1985; USEPA 1996). It was considered important to determine if dose rate also plays a role in UV inactivation. This study determined the influence of how UVB dose was delivered, the exposure–dose rate relationship, on MS2 inactivation in optically clear water.

## UVB inactivation of *E. coli* and the effect of temperature

The current study explored the UVB inactivation of *E. coli* at two temperatures encompassing the range frequently measured in HRAPs operated in South Australia, 20 °C and 30 °C, in buffered (pH 7.5) RO water. No *E. coli* inactivation was recorded in the dark incubated controls at either temperature over the 48-h incubation period (Table 3). This result was similar to that of MS2.

Generally, the *E. coli* inactivation rate increased with the increase of UVB dose rate. However, a few exceptions were recorded. At 20 °C, an increase in dose rate from 1 Wm$^{-2}$ to 2 Wm$^{-2}$ resulted in no significant difference in $K_i$ ($p = 0.441$). Similarly, at 30 °C, an increase from 2 Wm$^{-2}$ to 3 Wm$^{-2}$ resulted in no significant increase in $K_i$ ($p = 0.857$). As with the data for MS2, no statistically significant ($p < 0.05$) difference in *E. coli* inactivation rates was observed between 20 °C and 30 °C in the range of UVB dose rates investigated (Figure 3). The *E. coli* inactivation rates ($K_i$) at five UVB dose rates were modelled using log$_{10}$-linear regression: the equations of the regression lines at 20 °C and 30 °C are shown in Equations (5) and (6) in the supplementary information, respectively (available with the online version of this paper). As no significant difference was found between the two lines, a linear regression analysis was performed on the combined $K_i$ values obtained at 20 °C and 30 °C (as shown in Equation (7) in the supplementary

information, available online). This log10-linear model was applicable for temperatures between 20 °C and 30 °C.

Results presented here demonstrate, that for all incubation temperatures studied, the MS2 LRV recorded at the same dose (Jm$^{-2}$) was dependent on how that dose was delivered, that is, the dose rate (Wm$^{-2}$) – exposure time relationship. A 'dose rate-limited' and 'dose rate-saturated' phase of the LRV-UVB dose rate curve was observed at all UVB doses except 6,126 Jm$^{-2}$ incubated at 20 °C and 30 °C. The term dose rate-limited described the part of the LRV – dose rate curve where LRV increases with increasing dose rate while the UVB dose remains constant. This phase occurred between 0.5 and 3 Wm$^{-2}$ at all five UVB doses. The UVB dose rate limited inactivation phase may reflect the relationship between the photon fluence rate, the impact on RNA target sites and the rate of formation of
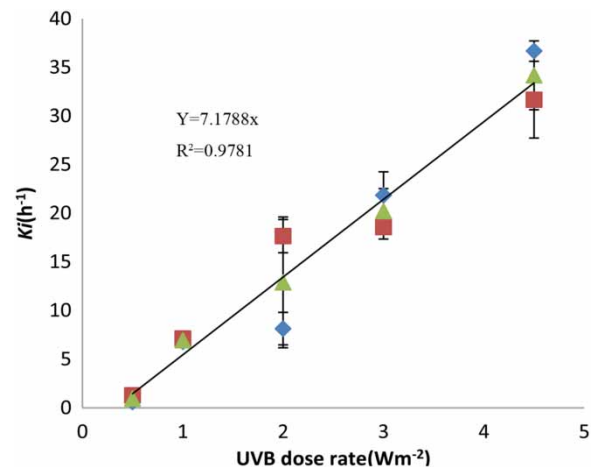


**Figure 3** | Comparison of *E. coli* inactivation rate ($K_i$) in buffered 7.5 pH RO water at 20 °C (♦) and 30 °C (■) the mean of the $K_i$ at 20 °C and 30 °C (▲) and linear regression of the mean $K_i$ (h$^{-1}$) at UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm$^{-2}$.

**Table 3** | *E. coli* inactivation rates $K_i$ (h$^{-1}$) in buffered pH 7.5 RO water at UVB dose rates of 0.5, 1, 2, 3 and 4.5 Wm$^{-2}$ (at a constant dose of 86.49 kJ m$^{-2}$) at 20 °C and 30 °C

| Temperature (°C) | UVB dose rate (Wm$^{-2}$) | Incubation time (h) | $K_i$ (h$^{-1}$) | SD | $R^2$ | n |
|---|---|---|---|---|---|---|
| 20 | Dark Control | 48 | 0.00 | 0.00 | 0.0012 | 12 |
|  | 0.5 | 48.05 | 0.6 | 0.09 | 0.9817 | 27 |
|  | 1 | 24.02 | 6.78 | 0.37 | 0.9948 | 27 |
|  | 2 | 12.01 | 8.14 | 1.67 | 0.9469 | 21 |
|  | 3 | 8.01 | 21.85 | 2.41 | 0.9856 | 18 |
|  | 4.5 | 5.34 | 36.69 | 0.31 | 1.000 | 12 |
| 30 | Dark Control | 48 | 0.00 | 0 | 0.01 | 12 |
|  | 0.5 | 48.05 | 1.28 | 0.39 | 0.9058 | 21 |
|  | 1 | 24.02 | 7.13 | 0.51 | 0.9951 | 21 |
|  | 2 | 12.01 | 17.64 | 1.71 | 0.9899 | 21 |
|  | 3 | 8.01 | 18.57 | 1.23 | 0.9935 | 18 |
|  | 4.5 | 5.34 | 31.68 | 3.95 | 0.9935 | 12 |

*Notes: $K_i$ (h$^{-1}$) mean ± SD was obtained from a log$_{10}$-linear + tail model using the GInaFiT model (Geeraerd *et al.* 2005) for which the individual $R^2$ values are shown together with n, the number of data points included in the model.*

pyrimidine dimers (Zhang *et al.* 2017). The onset of UVB dose rate saturated MS2 inactivation may be temperature dependent since it occurred at dose rates $>3\,\mathrm{Wm^{-2}}$ at $10\,^{\circ}\mathrm{C}$ and $20\,^{\circ}\mathrm{C}$ compared to $>1\,\mathrm{Wm^{-2}}$ at $30\,^{\circ}\mathrm{C}$. These results suggest that both the dose $(\mathrm{Wm^{-2}})$, dose rates $(\mathrm{Jm^{-2}})$ and temperature should be reported for all UV disinfection-related research.

Related research has been performed for UVC disinfection (Sommer *et al.* 1998). They found that the LRV of MS2 did not change obviously between three UVC dose rates $(0.02\,\mathrm{Wm^{-2}},\ 0.2\,\mathrm{Wm^{-2}}$ and $2\,\mathrm{Wm^{-2}})$ when UVC doses were maintained, by varying exposure time, at $100\,\mathrm{Jm^{-2}}$, $300\,\mathrm{Jm^{-2}}$ or $500\,\mathrm{Jm^{-2}}$ in sterile 0.85% saline. This finding was consistent with the results for the low UVB dose $(6{,}126\,\mathrm{Jm^{-2}})$ delivered by five different UVB dose rates at

either $20\,^{\circ}\mathrm{C}$ or $30\,^{\circ}\mathrm{C}$ reported here. In contrast, Liu & Zhang (2006) observed that a higher UVC dose caused more MS2 LRV than the same dose achieved using a lower dose rate for a longer time. In their study, the UVC dose rates selected were 0.08, 1.0 and $1.8\,\mathrm{Wm^{-2}}$, to achieve a constant dose of $400\,\mathrm{Jm^{-2}}$. The results for UVB presented here are consistent with those results obtained for UVC.

## Interaction between UVB dose rate $(\mathrm{Wm^{-2}})$ and UVB dose $(\mathrm{Jm^{-2}})$ on *E. coli* UVB inactivation

*E. coli* was found to be more sensitive to UVB inactivation than MS2 in buffered pH 7.5 RO water. Consequently, only one LRV data point was available for either of the high UVB doses, $62{,}231\,\mathrm{Jm^{-2}}$ or $86{,}490\,\mathrm{Jm^{-2}}$ (Figure 4),
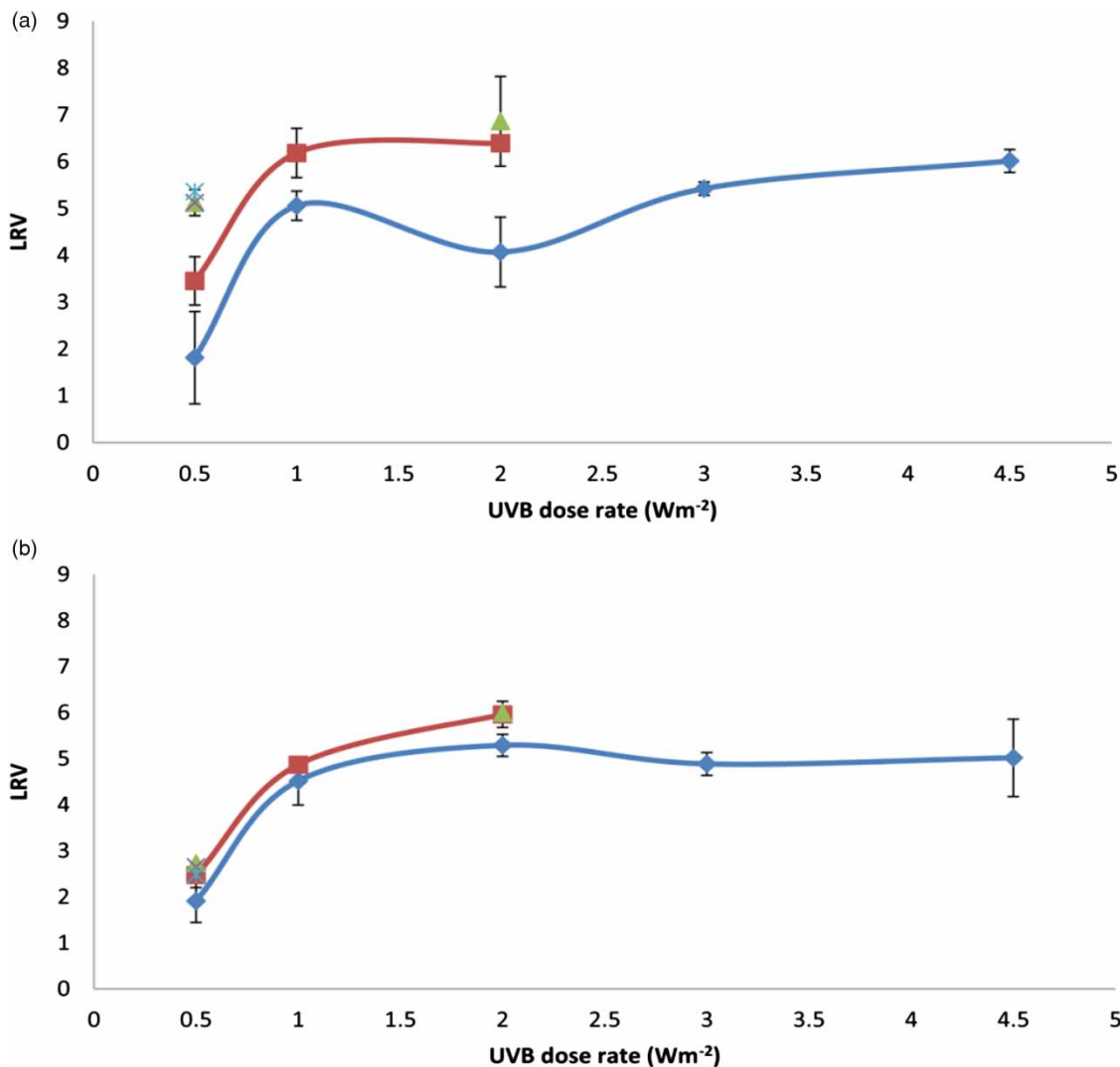


**Figure 4** │ *E. coli* $\log_{10}$ reduction value (LRV) in buffered pH 7.5 RO water, using five UVB dose rates–time combinations: 0.5, 1, 2, 3 and 4.5 $\mathrm{Wm^{-2}}$, to achieve five UVB doses: 6,126 (◆), 22,049 (■), 37,973 (▲), 62,231 (✕) and 86,490 (✻) $\mathrm{Jm^{-2}}$ at (a) 20 °C and (b) 30 °C.

since all the *E. coli* were inactivated within the first exposure period. Similarly, at 22,049 Jm$^{-2}$, the response to only three UVB dose rates (Wm$^{-2}$) could be recorded. The sensitivity of *E. coli* to UVB inactivation was such that only at the lowest UVB dose, 6,126 Jm$^{-2}$, LRVs were able to be determined at all five dose rates.

These limitations make data interpretation more difficult and less conclusive. *E. coli* LRV significantly ($p < 0.001$) increased with increasing UVB dose (Jm$^{-2}$). However, as recorded for MS2, the dose rate (Wm$^{-2}$)–exposure time combination used to achieve the dose (Jm$^{-2}$) influenced the *E. coli* LRV. At both temperatures of 20 °C and 30 °C and UVB doses of 22,049 Jm$^{-2}$ and 6,126 Jm$^{-2}$, the *E. coli* LRV increased rapidly when the dose rate used to achieve the same UVB dose was increased from 0.5 Wm$^{-2}$ to $\geq 1$ Wm$^{-2}$; this was termed 'dose rate-limited'. At other doses, the *E. coli* LRV plateaued at dose rates $> 2$ Wm$^{-2}$, a 'dose rate-saturated' phase. These results were similar to those recorded for MS2 over a greater range of UVB doses.

The manner in which a constant UVB dose was delivered, that is, the UVB dose rate–exposure time combination also significantly influenced *E. coli* disinfection. Exposed to the same UVB doses and dose rates, it is obvious that *E. coli* was more sensitive to UVB irradiance than MS2; exposed to 4.5 Wm$^{-2}$, the $K_i$ of *E. coli* was an order of magnitude higher than the $K_i$ of MS2. This may be a consequence of both the larger physical size and greater amount of genetic material in *E. coli* compared to MS2. These characteristics may result in *E. coli* receiving more photons, so that the pyrimidine dimer is more readily formed on *E. coli*'s DNA. A similar conclusion has been supported by much UV-related research (Bolton 2011; Fisher *et al.* 2012; Theitler *et al.* 2012).

We are unaware of any other study which has systematically assessed and modeled the effect of environmentally relevant UVB doses, dose rates and temperatures on the MS2 and *E. coli* UVB inactivation rates and log$_{10}$ reduction values. Two phases were identified for the UVB inactivation and LRV curves for both organisms: a UVB dose rate limited inactivation phase and a dose rate saturation inactivation phase. This initial study characterizes the UVB inactivation of two important indicator organisms in optically clear, buffered (pH 7.5) water. This, together with further research considering the effect of water column attenuation of UVB, will enable the development of a more accurate pathogen inactivation model in natural wastewater treatment systems, e.g. HRAPs and WSPs.

## CONCLUSIONS

In optically clear, buffered (pH 7.5), RO water, ambient temperature had a significant effect on UVB inactivation of MS2 between 10 °C and 30 °C. Two phases were identified for the UVB inactivation and LRV curves at each of the five UVB doses for both *E. coli* and MS2: a UVB dose rate limited inactivation phase and a dose rate saturated inactivation phase. The results suggest that both the dose (Wm$^{-2}$) and dose rates (Jm$^{-2}$) should be reported for all UV disinfection-related research. This research contributes to a better understanding on natural UVB disinfection, which improves the accuracy of HRAPs pathogen disinfection performance to a large extent.

## ACKNOWLEDGEMENTS

## REFERENCES

Berney, M., Weilenmann, H. U., Ihssen, J., Bassin, C. & Egli, T. 2006 Specific growth rate determines the sensitivity of *Escherichia coli* to thermal, UVA, and solar disinfection. *Applied and Environmental Microbiology* **72** (4), 2586–2593.

Bolton, N. F. 2011 *Photoinactivation of Indicator Micro-Organisms and Adenovirus in Sunlit Waters*. PhD, Department of Environmental Health, Flinders University, Adelaide, SA, Australia.

Bolton, N. F., Cromar, N. J., Hallsworth, P. G. & Fallowfield, H. J. 2010 A review of the factors affecting sunlight inactivation of micro-organisms in waste stabilisation ponds: preliminary results for enterococci. *Water Science and Technology* **61** (4), 885–890.

Bolton, N. F., Cromar, N. J., Buchanan, N. A. & Fallowfield, H. J. 2011 Variations in sunlight attenuation in waste stabilisation ponds and environmental waters. In: *9th IWA Specialist Group on Waste Stabilisation Ponds*, Adelaide, SA, Australia.

Bunsen, R. & Roscoe, H. E. 1857 Photo-chemical researches. Part I. Measurement of the chemical action of light. *Philosophical Transactions of the Royal Society of London* **147**, 355–380.

Chan, Y. & Killick, E. 1995 The effect of salinity, light and temperature in a disposal environment on the recovery of *E. coli* following exposure to ultraviolet radiation. *Water Research* **29** (5), 1373–1377.

Chang, J. C. 1985 UV inactivation of pathogenic and indicator microorganisms. *Applied and Environmental Microbiology* **49** (6), 1361.

Craggs, R. J., Zwart, A., Nagels, J. W. & Davies-Colley, R. J. 2004 Modelling sunlight disinfection in a high rate pond. *Ecological Engineering* **22** (2), 113.

Diamond, S. A., Trenham, P. C., Adams, M. J., Hossack, B. R., Knapp, R. A., Stark, S. L., Bradford, D., Corn, P. S., Czarnowski, K. & Brooks, P. D. 2005 Estimated ultraviolet radiation doses in wetlands in six national parks. *Ecosystems* **8** (5), 462–477.

Fisher, M. B., Iriarte, M. & Nelson, K. L. 2012 Solar water disinfection (SODIS) of *Escherichia coli, Enterococcus* spp., and MS2 coliphage: effects of additives and alternative container materials. *Water Research* **46** (6), 1745–1754.

Geeraerd, A. H., Geeraerd, V. P., Valdramidis, J. F. & Van, I. 2005 GInafit, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology* **102** (1), 95–105.

Hashimoto, K., Irie, H. & Fujishima, A. 2005 TiO$_2$ photocatalysis: a historical overview and future prospects. *Japanese Journal of Applied Physics* **44** (12R), 8269.

Hijnen, W., Beerendonk, E. & Medema, G. J. 2006 Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: a review. *Water Research* **40** (1), 3–22.

Kadir, K. & Nelson, K. L. 2014 Sunlight mediated inactivation mechanisms of *Enterococcus faecalis* and *Escherichia coli* in clear water versus waste stabilization pond water. *Water Research* **50**, 307–317.

Kohn, T. & Nelson, K. L. 2006 Sunlight-mediated inactivation of MS2 coliphage via exogenous singlet oxygen produced by sensitizers in natural waters. *Environmental Science & Technology* **41** (1), 192–197.

Li, W., Li, M., Wen, D. & Qiang, Z. 2018 Development of economical-running strategy for multi-lamp UV disinfection reactors in secondary water supply systems with computational fluid dynamics simulations. *Chemical Engineering Journal* **343**, 317–323.

Liu, W.-j. & Zhang, Y.-j. 2006 Effects of UV intensity and water turbidity on microbial indicator inactivation. *Journal of Environmental Sciences* **18** (4), 650–653.

Mbonimpa, E. G., Vadheim, B. & Blatchley, E. R. 2012 Continuous-flow solar UVB disinfection reactor for drinking water. *Water Research* **46** (7), 2344–2354.

Mirman, D. 2014 *Growth Curve Analysis and Visualization Using R*. Taylor & Francis, Boca Raton, FL, USA.

Murakami, E. G., Jackson, L., Madsen, K. & Schickedanz, B. 2006 Factors affecting the ultraviolet inactivation of *Escherichia coli* K12 in apple juice and a model system. *Journal of Food Process Engineering* **29** (1), 53–71.

Noble, R. T., Lee, I. M. & Schiff, K. C. 2004 Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. *Journal of Applied Microbiology* **96** (3), 464–472.

Sommer, R., Haider, T., Cabaj, A., Pribil, W. & Lhotsky, M. 1998 Time dose reciprocity in UV disinfection of water. *Water Science and Technology* **38** (12), 145–150.

Theitler, D., Nasser, A., Gerchman, Y., Kribus, A. & Mamane, H. 2012 Synergistic effect of heat and solar UV on DNA damage and water disinfection of *E. coli* and bacteriophage MS2. *Journal of Water and Health* **10** (4), 605–618.

Torres, E. F., González-M, G., Klotz, B. & Rodrigo, D. 2016 Effects of high hydrostatic pressure and temperature increase on *Escherichia coli* spp. and pectin methyl esterase inactivation in orange juice. *Food Science & Technology International* **22** (2), 173.

USEPA 1996 *Ultraviolet Light Disinfection Technology in Drinking Water Application–an Overview*. EPA 811-R-96-002, Office of Ground Water and Drinking Water, Washington, DC, USA.

Vermeulen, N., Keeler, W. J., Nandakumar, K. & Leung, K. T. 2008 The bactericidal effect of ultraviolet and visible light on *Escherichia coli*. *Biotechnology and Bioengineering* **99** (3), 550–556.

Wegelin, M., Canonica, S., Mechsner, K., Fleischmann, T., Pesaro, F. & Metzler, A. 1994 Solar water disinfection: scope of the process and analysis of radiation experiments. *Aqua* **43** (4), 154–169.

Young, P., Buchanan, N. & Fallowfield, H. 2016 Inactivation of indicator organisms in wastewater treated by a high rate algal pond system. *Journal of Applied Microbiology* **121** (2), 577–586.

Zhang, S., Ye, C., Lin, H., Lv, L. & Yu, X. 2015 UV disinfection induces a VBNC state in *Escherichia coli* and *Pseudomonas aeruginosa*. *Environmental Science & Technology* **49** (3), 1721–1728.

Zhang, C. M., Xu, L. M., Wang, X. C., Zhuang, K. & Liu, Q. Q. 2017 Effects of ultraviolet disinfection on antibiotic-resistant *Escherichia coli* from wastewater: inactivation, antibiotic resistance profiles and antibiotic resistance genes. *Journal of Applied Microbiology* **123** (1), 295–306.

Zyara, A. M., Torvinen, E., Veijalainen, A. M. & Heinonen-Tanski, H. 2016 The effect of UV and combined chlorine/UV treatment on coliphages in drinking water disinfection. *Journal of Water and Health* **14** (4), 640–649.