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Role of Temperature and Suwannee River Natural Organic Matter on Inactivation Kinetics of Rotavirus and Bacteriophage MS2 by Solar Irradiation

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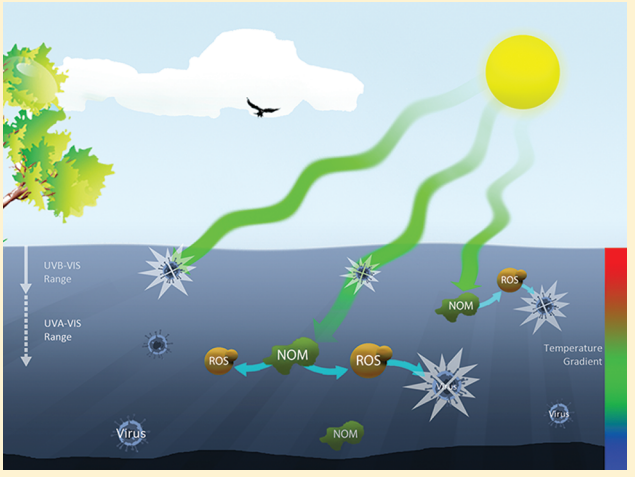
Supporting Information

ABSTRACT: Although the sunlight-mediated inactivation of viruses has been recognized as an important process that controls surface water quality, the mechanisms of virus inactivation by sunlight are not yet clearly understood. We investigated the synergistic role of temperature and Suwannee River natural organic matter (SRNOM), an exogenous sensitizer, for sunlight-mediated inactivation of porcine rotavirus and MS2 bacteriophage. Upon irradiation by a full spectrum of simulated sunlight in the absence of SRNOM and in the temperature range of 14−42 °C, high inactivation rate constants, k_{obs}, of MS2 ($k_{obs} \leq 3.8\ h^{-1}$ or 1-$\log_{10}$ over 0.6 h) and rotavirus ($k_{obs} \leq 11.8\ h^{-1}$ or ∼1-$\log_{10}$ over 0.2 h) were measured. A weak temperature (14−42 °C) dependence of k_{obs} values was observed for both viruses irradiated by the full sunlight spectrum. Under the same irradiation condition, the presence of SRNOM reduced the inactivation of both viruses due to attenuation of lower wavelengths of the simulated sunlight. For rotavirus and MS2 solutions irradiated by only UVA and visible light in the absence of SRNOM, inactivation kinetics were slow ($k_{obs} < 0.3\ h^{-1}$ or <1-$\log_{10}$ unit reduction over 7 h) and temperature-independent for the range considered. Conversely, under UVA and visible light irradiation and in the presence of SRNOM, temperature-dependent inactivation of MS2 was observed. For rotavirus, the SRNOM-mediated exogenous inactivation was only important at temperatures >33 °C, with low rotavirus k_{obs} values ($k_{obs} \approx 0.2\ h^{-1}$; 1-$\log_{10}$ unit reduction over 12 h) for the temperature range of 14−33 °C. These k_{obs} values increased to $0.5\ h^{-1}$ at 43 °C and $1.5\ h^{-1}$ (1-$\log_{10}$ reduction over 1.6 h) at 50 °C. While SRNOM-mediated exogenous inactivation of MS2 was triggered by singlet oxygen, the presence of hydrogen peroxide was important for rotavirus inactivation in the 40−50 °C range.



INTRODUCTION

Surface water is an important resource for irrigation, drinking water, and recreation. Runoff from both agricultural and urban land can lead to surface water contamination by bacterial and viral pathogens.[1−5] In California, surface water contaminated by stormwater runoff has been linked to an increased health risk for swimmers.[6] A 9-year study of water quality in the Great Lakes found culturable pathogenic viruses present in wastewater discharged to Lake Michigan and the influent water of nearby drinking water treatment plants.[7] Viral nucleic acids of norovirus, adenovirus, and astrovirus have been found in urban water catchments, which are used as drinking water sources for millions of people in Singapore.[3,4] The presence of viruses in drinking water sources is notably troublesome because, as compared to bacteria, viruses are more difficult to remove and inactivate by conventional water treatment technologies.[8−10] In places without centralized water treatment facilities, surface and rainwater may be used for drinking after treatment by sunlight or heat.[11] Furthermore, wastewater may be treated by a system of stabilization ponds, which relies on solar irradiation for pathogen disinfection, and then used for irrigation.[12−14] Despite the importance of microbial surface water quality, a clear understanding of pathogen inactivation mechanisms in surface water is not yet available, particularly for enteric viruses. Field studies have linked the persistence of pathogens, including enteric viruses, in coastal water with sunlight irradiation.[1,2] Other studies have shown that sunlight disinfection is important in sunlit surface waters. Under various

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conditions tested, solar disinfection studies have reported higher inactivation rates for bacterial indicators than for virus indicators.[15,16] These findings stress the need for a better understanding of solar disinfection mechanisms of viruses.

Three different mechanisms have been identified in the solar disinfection of microorganisms: direct, indirect endogenous, and indirect exogenous inactivation.[15,17] The first mechanism involves direct damage of the genome by light in the UVB (280−320 nm) range and the formation of photoproducts that block genome replication.[18,19] In addition, direct UVB damage to the proteins of adenovirus has also been reported.[20] Indirect endogenous inactivation is initiated by light absorption via sensitizers associated with the microorganisms themselves (e.g., riboflavins, porphyrin).[21−25] Electron or energy transfer from the excited sensitizers to dissolved oxygen leads to the formation of reactive oxygen species (ROS) that damage internal targets (i.e., nucleic acids, proteins).[19] Indirect exogenous inactivation causes photo-oxidative damage of microorganisms by ROS formed by the external sensitizers such as natural organic matter (NOM) in solution.[26,27] Both endogenous and exogenous inactivation can be initiated by light in the UVB range as well as by higher wavelength light.

UVB radiation is indisputably effective for disinfection of viruses, including enteric viruses and bacteriophages used as pathogen indicators.[27,28] Some F-RNA, F-DNA, and somatic phages were shown to only be inactivated by the UVB portion of the sunlight spectrum.[16,17] However, a number of RNA phages are also effectively inactivated by the indirect exogenous mechanism.[16,17,27,28] NOM and trace metals have been found to serve as exogenous sensitizers facilitating inactivation of bacteriophages.[26,27,29] Specifically, among the ROS produced by the irradiation of NOM, singlet oxygen ($^1O_2$) was the most significant species causing the inactivation of MS2 bacteriophage.[26,27] In the presence of trace metals, such as copper and iron and hydrogen peroxide (H_2O_2), photo-Fenton-like processes efficiently formed hydroxyl radicals (·OH) capable of inactivating MS2.[29] Thus, exogenous inactivation mediated by the ubiquitous sensitizers NOM or iron could serve as an important disinfection process of viruses in surface waters.

Rotavirus has been recognized as the most common cause of acute infectious gastroenteritis in children;[30] nevertheless, all age groups are affected worldwide.[31] Besides infecting humans, rotavirus also infects the agriculturally important swine and calves among others.[32] Among seven infectious serogroups of rotavirus, labeled A−G, only groups A−C are human pathogens, with group A being the primary pathogenic type for humans worldwide and responsible for the majority of outbreaks.[30] These serogroups are distinguished by antigenic differences within the virus core and by migration of ds RNA segments;[30] however, each serogroup may share the same cellular receptor.[33] Rotavirus is highly infectious and spreads via the fecal-oral route. Infected individuals shed rotavirus at high concentrations (10^{10}−10^{11} virions per gram of feces) for up to 2 weeks.[30] Rotavirus infectivity can be retained for several days in water environments at temperatures up to 40 °C and for ≥1 h in the pH range of 3−10.[30,34−37] The rotavirus minimal infective dose has been reported to be as little as one cell culture-infective unit,[38] but higher infective doses have also been reported.[39] While the main route of rotavirus transmission is person-to-person contact, all documented rotavirus waterborne outbreaks have been associated with direct fecal contamination of a water supply or improper water treatment.[31] Because of its resilient characteristics and prevalence, particularly in the developing world,[40] rotavirus transmission via water has been recognized as a potentially significant public health problem.[38] The objectives of this study are to investigate the synergistic roles of temperature, sunlight, and SRNOM on inactivation mechanisms of group A porcine rotavirus, and to compare its inactivation to that of the commonly used viral surrogate MS2.

MATERIALS AND METHODS

Virus inactivation experiments were conducted in solutions irradiated by either full spectrum simulated sunlight or partial spectrum without UVB. The investigated temperature range was 14−35 °C, which covered the range of surface water temperatures under sunlight conditions.[41] We further increased the temperature to 60 °C, at which a previous study found complete inactivation of rotavirus by heat.[35] Suwannee River NOM (SRNOM) was used for a model sensitizer because it is well-characterized and has been used previously in photolysis studies.[42,43] Inactivation experiments with quenchers, which selectively remove ROS, and experiments with D_2O, which reduce singlet oxygen quenching as compared to H_2O, were also conducted to determine the radicals responsible for rotavirus inactivation. Concentrations of singlet oxygen and OH radicals and accumulation rates of hydrogen peroxide were also determined. Methods for light screening calculations, radical concentration measurements, quenching experiments, aggregation determination, and trace metal analysis can be found in the Supporting Information.

Model Viruses. MS2 bacteriophage (ATCC 15597-B1) was obtained from the American Type Culture Collection and was replicated and purified as described elsewhere.[44] Briefly, MS2 was propagated in *Escherichia coli* (ATCC 15597) and further purified by sequential centrifugation (Eppendorf centrifuge 5416) at 5000 rpm ($g \times 100$) for 15 min at 20 °C and microfiltered to remove *E. coli* cell debris. Subsequently, the filtered MS2 stock was concentrated on a Millipore 100 kDa membrane (Koch Membranes, U.S.) and rinsed with sterilized 1 mM NaCl (Fisher) solution in an ultrafiltration unit (Whatman Nucleopore, U.S.). The final MS2 stock, with a concentration of $\sim 10^{11}$ plaque forming units (PFU) per mL, was stored at 4 °C in sterilized 1 mM NaCl solution. MS2 enumeration was performed following the double agar layer procedure.[45] Group A porcine rotavirus OSU strain (ATCC # VR-892) was propagated in embryonic African green monkey kidney cells (MA-104 cells) and was extracted from the cell culture as described elsewhere.[32] Rotavirus was purified following the same protocol as for MS2, except the microfiltration through the 0.2 μm membrane was not performed. To prevent outer capsid protein denaturation,[46] a solution of 1 mM NaCl and 0.5 mM $CaCl_2$ (Fisher) was used for concentrating rotavirus with 100 kDa membrane. The final purified rotavirus stock concentration of $\sim 10^6$ focus forming units (FFU) per mL was stored in the dark at 4 °C. Samples were enumerated by infectivity assay[33] with concentrations of rotavirus reported as focus forming units per mL (FFU/mL). A brief description of rotavirus infectivity and focus forming assay is included in the Supporting Information.

Model Aquatic Natural Organic Matter (NOM). Suwannee River NOM (SRNOM) was obtained from the International Humic Substances Society. The procedure for NOM solution preparation was described elsewhere.[47] Briefly, SRNOM was dissolved in a solution containing 1 mM NaCl and 2 mM $NaHCO_3$ (Fisher). The final SRNOM solution at pH 7.2 was

filtered through a 0.22 μm acetate membrane and stored at 4 °C. The total organic carbon concentration of 230 mg/L (reported as mg C/L) for the final SRNOM stock solution was measured at the Illinois Sustainable Technology Center.

Experimental Setup for Virus Inactivation. Solar disinfection experiments were conducted using an Atlas Suntest(r) XLS+ photosimulator (Chicago, IL) equipped with a xenon arc lamp, which irradiates light with wavelength from 280 nm and above. The solar simulator was also fitted with a filter (Atlas MTS, Cat. 56052372), which cuts off most, but not all, of the irradiance below 320 nm. This Atlas filter was in place throughout all irradiation experiments conducted, and it was the only filter used in the full spectrum (280−700 nm) irradiation experiments. Because of the UVB bleeding of the Atlas filter, a second UVB filter (Newport, FSQ-WG320) with a 320 nm cutoff was placed atop each reactor testing UVA and visible light (320−700 nm) effects. A calibrated StellarNet Inc. spectrometer was used to measure the irradiance of the solar simulator from 280 to 700 nm. The solar simulator intensity was set to 400 W m^{-2}. The fraction of the UVB (280−320 nm) irradiance measured with and without the Newport 320 nm cutoff filter was 4.71% and 6.24%, respectively. For comparison, the fraction of the UVB irradiance of natural sunlight measured at noon on March 5, 2010 during a clear day in Urbana, IL was 5.86%. The measured irradiance did not change with or without the Newport 320 nm cutoff filter.

All reactors were painted black to avoid light reflection and immersed in a circulating water bath. All solutions were stirred by a Variomag electronic stirrer set to 130 rpm. For MS2 inactivation, 50 mL pyrex beakers with 40 mL of 1 mM sodium bicarbonate buffer, SRNOM (0−20 mg C/L), and an initial MS2 concentration of 10^8 plaque forming units (PFU) per mL were used. One milliliter sample aliquots were collected in 1.7 mL centrifuge tubes at regular intervals and stored in the dark until enumeration (normally 2−12 h from sample collection).

For rotavirus inactivation experiments, a similar protocol was used. A 0.5−1 mL aliquot of the rotavirus stock solution was added to each 10 mL pyrex beaker, achieving a 10^4−10^5 FFU/mL concentration in each reactor. A 200 μL sample aliquot was collected in 1.5 mL centrifuge tubes at regular intervals and stored in the dark until the samples were enumerated (within 2−12 h).

pH readings of each reactor were taken immediately before and after irradiation. The pH remained stable (7.6−8.0 ± 0.2) for all conditions tested. A duplicate reactor without the virus spike was included with each experiment to take temperature readings during each sample acquisition. The same sample volume was also withdrawn from the duplicate reactor, so that all reactors had the same volume throughout the experiment.

Kinetic Data Analysis. The pseudo-first-order inactivation rate, k_{obs} (h^{-1}), was obtained by determining the slope of ln(PFU or FFU/mL) versus time (h) plots. Linear regression analysis was used to calculate the k_{obs} value and the corresponding 95% confidence interval for a single condition. Linear regression analysis was also used to determine whether the slopes of the lines in the inactivation kinetic and k_{obs} versus temperature plots were significantly different from a slope of zero ($p < 0.05$). A multiple linear regression analysis[48] was used to compare if the slopes of the inactivation kinetic and k_{obs} versus temperature plots for different conditions were significantly different ($p < 0.05$) from each other. A single sample was collected from each reactor at a given time. For each sample, appropriate dilutions were made, and each dilution was plated twice on either wells (for rotavirus) or Petri dishes (for MS2).

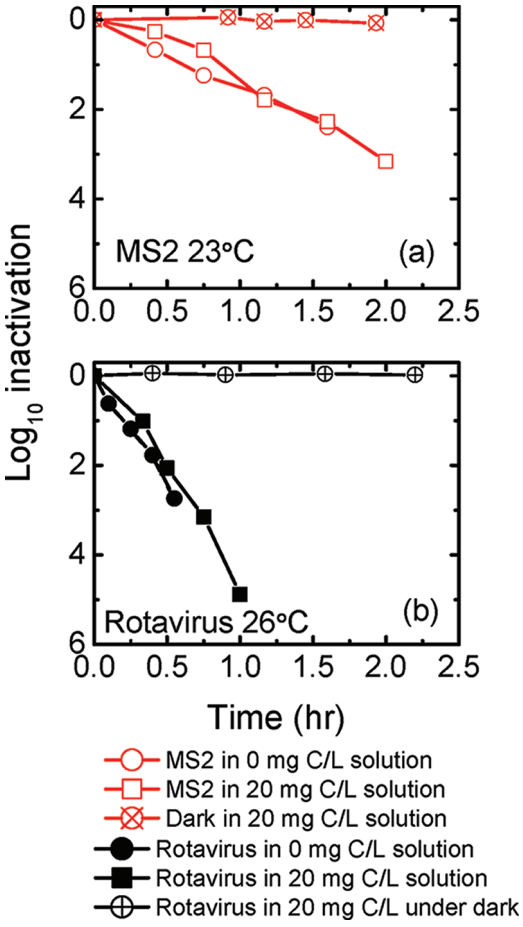

Figure 1. Full sunlight spectrum inactivation of MS2 (a) and rotavirus (b) at 23 and 26 °C, respectively, as a function of time in the presence and absence of SRNOM in 1 mM sodium bicarbonate buffer. The plots were corrected for light screening.

Counts in duplicate were averaged and used for further analysis. Most conditions were tested multiple times in separate experiments, with similar trends (see Table S1).

RESULTS

Full Sunlight Spectrum Inactivation at 23−26 °C. UVB has been reported to be the most lethal portion of the solar spectrum for most indicators of waterborne pathogens;[16,49] accordingly, rapid inactivation of both rotavirus and MS2 was observed in bicarbonate-buffered solutions irradiated by the full sunlight spectrum (Figure 1). UVB, however, is rapidly screened if NOM is present, and much slower inactivation kinetics were observed in solutions containing 20 mg C/L. When the inactivation rate constants were corrected for UVB light screening, statistically similar inactivation kinetics were observed in the presence and absence of SRNOM for both MS2 ($p = 0.37$) and rotavirus ($p = 0.83$) at 23 and 26 °C, respectively (Figure 1). This observation suggests that the SRNOM-mediated, indirect exogenous effects were negligible for MS2 and rotavirus inactivation by the full sunlight spectrum.

Dark and Endogenous Inactivation of MS2 and Rotavirus at 23−26 °C. The contribution of endogenous inactivation was investigated by irradiating virus solutions with the UVB portion removed from the full sunlight spectrum. This condition avoids

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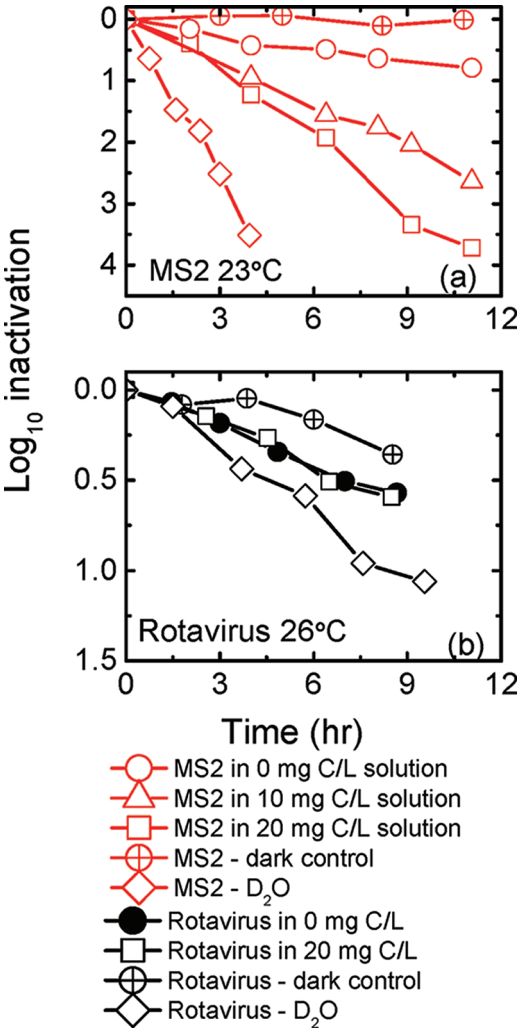

Figure 2. Inactivation kinetics for MS2 (a) and rotavirus (b) under UVA and visible light irradiation at 23 and 26 °C, respectively. All experiments were conducted in 1 mM sodium bicarbonate buffer. Except for the dark conditions, samples were irradiated with UVA and visible light. The plots have been corrected for light screening.

the confounding effects of direct inactivation. In the absence of SRNOM and exposed only to light in the UVA/visible range, MS2 and rotavirus showed slow and statistically similar (p = 0.86) endogenous inactivation kinetics ($k_{obs} \approx 0.16 \pm 0.03$ h^{-1}; ~0.5-log unit reduction over 7 h; see Figure 2a and b). This observed slow inactivation for MS2 and rotavirus irradiated with UVA and visible light is consistent with other previously reported studies with RNA viruses,[17,27] which appear to be resistant to endogenous inactivation. In the dark, neither MS2 nor rotavirus inactivation kinetics showed significant (p = 0.70−0.62) deviation from a slope of zero in the presence or absence of SRNOM over 10 h.

SRNOM-Mediated Exogenous Inactivation of MS2 and Rotavirus at 22−23 °C. As discussed above, the contribution of exogenous inactivation was insignificant in experiments using the full sunlight spectrum. However, as light in the UVB range is rapidly screened by NOM in solution, exogenous inactivation may become relevant at lower depths in a water column. For this reason, irradiation by UVA and visible light was also investigated. The inactivation kinetics of MS2 in solutions containing different concentrations of organic matter irradiated with light in the UVA/visible range is illustrated in Figure 2a. The SRNOM-mediated exogenous inactivation rate constants, k_{obs}, for MS2 increased with SRNOM concentrations (expressed as DOC) after light screening correction. Specifically, the MS2 k_{obs} values at [DOC] of 0, 10, and 20 mg C/L were 0.16 ± 0.03, 0.53 ± 0.5, and 0.91 ± 0.1 h^{-1}, respectively. Inactivation kinetics for these conditions were significantly different from each other (p values <0.01).

Previous studies have shown that among the ROS formed by irradiated humic acid, $^1O_2$ was the most important disinfectant for MS2.[27] The increased k_{obs} values for MS2 can be explained by the increased $^1O_2$ formation as a function of SRNOM concentration (Figure 2S in the Supporting Information). Evidence of the importance of $^1O_2$ for MS2 inactivation by SRNOM is given by experiments conducted in D_2O (Figure 2a). Water is a more efficient $^1O_2$ quencher than D_2O. Specifically, the quenching rates of $^1O_2$ in D_2O and H_2O are 1.6×10^4 and 2.5×10^5 s^{-1}, respectively.[50] Therefore, the $^1O_2$ concentration in D_2O should be higher than that in water. Indeed, in solution containing 20 mg C/L, the measured $^1O_2$ concentrations were $(24.5 \pm 3.4) \times 10^{-14}$ M in water to $(120 \pm 5) \times 10^{-14}$ M in D_2O. This 5-time increase in $^1O_2$ concentration resulted in an MS2 k_{obs} value 2.4-times higher in D_2O than in H_2O. The lower than expected 5-time increase in k_{obs} suggests that $^1O_2$ may only account for a part of the total inactivation of MS2. In the absence of SRNOM, no significant (p = 0.57) difference in inactivation kinetics was observed between the D_2O and H_2O conditions. Thus, the increase in k_{obs} value with increasing concentrations of $^1O_2$ formed by SRNOM is consistent with previous studies, suggesting its important role.[18]

No significant difference (p = 0.61) in rotavirus inactivation kinetics was observed in the presence or absence of SRNOM over 9 h. To confirm the lack of $^1O_2$-induced rotavirus inactivation, additional experiments were conducted in D_2O. In the presence of 20 mg C/L, the rotavirus k_{obs} value was 1.6 times higher in D_2O than in H_2O (p = 0.006 for comparison of kinetic data). This small increase in rotavirus k_{obs} value suggests that $^1O_2$ only minimally inactivates rotavirus at 23 °C and in the presence of 20 mg C/L SRNOM. Our results suggest that $^1O_2$ formed by NOM in sunlit surface waters at ambient temperatures is not expected to inactivate rotavirus because $^1O_2$ is present only at low steady-state concentrations ($<10^{-12}$ M).[51]

Role of Temperature on Inactivation of MS2 and Rotavirus. The temperature dependence of MS2 and rotavirus inactivation in the dark is plotted in Figure 3a and b, respectively. We performed linear regression analysis to determine if the slope of the k_{obs} values plotted against temperature differed from a slope of zero (p < 0.05). Because the slopes for temperature (14−42 °C) and dark inactivation rate constants for MS2 (p = 0.38) and rotavirus (p = 0.19) did not differ from a slope of zero, we concluded that there was no significant trend between temperature and k_{obs} values for both viruses in the dark. The inactivation rate constants remained low for both viruses ($k_{obs} \approx$ 0.05 h^{-1} or 0.25-$\log_{10}$ unit reductions in 10 h) from 14 to 42 °C. However, at 50 °C, the k_{obs} values for both MS2 and rotavirus increased to 0.76 ± 0.2 h^{-1} (~1-$\log_{10}$ reduction over 3 h) and 0.5 ± 0.2 h^{-1} (~1-$\log_{10}$ reduction over 5 h), respectively. At 60 °C, the MS2 k_{obs} values obtained for the dark and for the condition without SRNOM increased to ~6 h^{-1} (~1-$\log_{10}$ reduction over 0.36 h; data not shown) with no statistically different inactivation kinetics (p = 0.14). For rotavirus, when the temperature reached 60 °C, no infectious virus was detected

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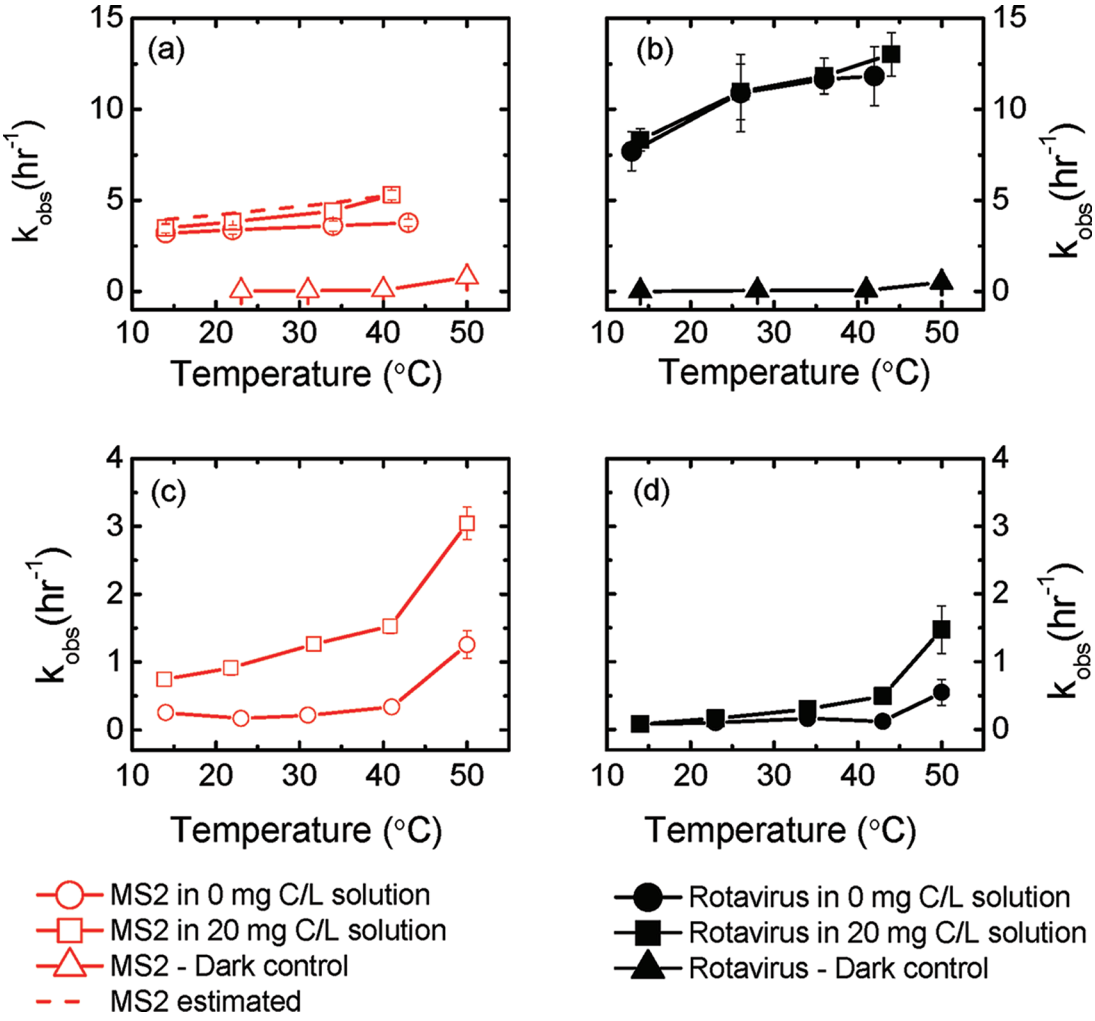

Figure 3. Inactivation rate constants as a function of temperature for MS2 irradiated with the full sunlight spectrum (a), rotavirus irradiated with the full sunlight spectrum (b), MS2 irradiated with UVA and visible light (c), and rotavirus irradiated with UVA and visible light (d). The plots have been corrected for light screening, and the error bars correspond to 95% confidence intervals. Only one set of the dark inactivation is shown for clarity.

from an initial sample of $10^{3.5}$ FFU/mL after 6 min of heat exposure (data not plotted) for any condition tested, including irradiation by the full sunlight spectrum and under dark conditions. Thus, the log reduction for this case was estimated to be 3.5 or k_{obs} of 30 h^{-1}. At a temperature equal to or above 60 °C, heat is expected to be the main factor for rotavirus inactivation. The rotavirus data presented here are in agreement with a previous study, wherein rotavirus was found to remain stable at 30 and 40 °C for at least 24 h in viral growth media. A 1.5-log unit reduction at 50 °C in 1 h (estimated k_{obs} of ~3.5 h^{-1}) and 3.25-log unit reduction at 60 °C in 5 min (est. k_{obs} of ~90 h^{-1}) were reported.[35]

For the full solar spectrum irradiation, MS2 solutions containing 0 mg C/L and 20 mg C/L were investigated in the temperature range of 14−42 °C. For these conditions, k_{obs} values were weakly but linearly correlated with temperature. Specifically, for solutions containing 0 and 20 mg C/L, the slopes of the regression lines for k_{obs} values and temperature were 0.02 (p = 0.0009) and 0.06 (p = 0.03), respectively (Figure 3a). For rotavirus irradiated by the full sunlight spectrum (Figure 3b) in solutions containing 0 and 20 mg C/L of SRNOM, the slopes of the regression lines for k_{obs} versus temperature (14−42 °C) were ~0.15 (p < 0.05). The inactivation kinetic plots for the two conditions aforementioned were not statistically different from each other (p = 0.85).

When MS2 was irradiated by only UVA and visible light (Figure 3c), the slope of the regression line for endogenous (i.e., without SRNOM) k_{obs} values and temperature (14−42 °C) did not significantly differ from a slope of zero (p = 0.46). The k_{obs} values obtained for MS2 solutions without SRNOM were <0.33 h^{-1} (<1-$\log_{10}$ unit reduction over ~7 h) in the temperature range of 14−42 °C. At 50 °C, the MS2 k_{obs} value increased to 1.26 ± 0.2 h^{-1} (~1-$\log_{10}$ unit reduction in 1 h).

For rotavirus, the UVA and visible light induced endogenous k_{obs} values (Figure 3d) remained low (k_{obs} < 0.2 h^{-1}; ~1-$\log_{10}$ unit reduction in 12 h) for the temperature range of 14−42 °C. The linear plots for the endogenous and dark k_{obs} values versus temperature (14−42 °C) were not statistically different from each other (p = 0.81). Under the same irradiation condition at 50 °C, the endogenous and dark kinetic plots were not statistically different from each other (p = 0.73) and measured a k_{obs} value of ~0.5 h^{-1}.

In the absence of UVB, the SRNOM-mediated exogenous (i.e., with 20 mg C/L of SRNOM) k_{obs} values for MS2 and rotavirus

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increased with temperatures from 14 to 50 °C (Figure 3c and d). For MS2, there was a linear relationship between temperatures from 14 to 42 °C and the SRNOM-mediated exogenous k_{obs} values (slope = 0.03; $p = 0.004$). The k_{obs} values due to SRNOM-mediated exogenous inactivation of MS2 increased from 1.5 ± 0.1 h^{-1} at 42 °C to 3 ± 0.2 h^{-1} at 50 °C and to 18.9 ± 1 h^{-1} (~1-log_{10} unit reduction in 0.13 h) at 60 °C.

For rotavirus under UVA and visible light irradiation, the linear plots obtained by plotting the endogenous and SRNOM-mediated exogenous k_{obs} values against temperature from 14 to 33 °C were not statistically different from each other ($p = 0.29$). For rotavirus, the k_{obs} values due to SRNOM-mediated exogenous inactivation increased from 0.3 ± 0.06 h^{-1} at 34 °C to 0.5 ± 0.05 h^{-1} at 42 °C and 1.5 ± 0.3 h^{-1} (~1-log_{10} unit reduction in 1.5 h) at 50 °C. Comparing the k_{obs} values obtained from solutions irradiated by UVA and visible light with and without SRNOM at 50 °C, the SRNOM-mediated exogenous conditions resulted in higher inactivation rates (1.5 vs 0.5 h^{-1}). Thus, this increased inactivation of rotavirus at 50 °C in the presence of SRNOM is due to the synergistic effects of heat, sunlight irradiation, and NOM. To our knowledge, this is the first study to report the importance of high temperature (i.e., >25 °C) for sunlight-mediated inactivation of rotavirus.

Quenching Experiments at 50 °C for Rotavirus. Quenching tests were conducted at 50 °C to identify which ROS was responsible for the inactivation of rotavirus by exogenous effects (see Table 1S). Singlet oxygen was investigated first, as it has been shown to be responsible for MS2 inactivation. At 50 °C and 20 mg C/L (Table 1S), the inactivation kinetics obtained in solutions with and without 50 mM L-histidine, a quencher for $^1O_2$, were not significantly different ($p = 0.58$). Also, for this same condition, the inactivation kinetics obtained in D_2O and H_2O were not statistically different ($p = 0.78$). Thus, the experimental results suggest that rotavirus inactivation at 50 °C is not primarily due to damage by $^1O_2$.

Quenching experiments with 200 U/mL catalase were conducted at 50 °C to determine the role of H_2O_2 on exogenous rotavirus inactivation (Table 2S). In the presence of catalase and 20 mg C/L, the rotavirus k_{obs} value decreased from 1.5 ± 0.3 to 0.5 ± 0.3 h^{-1}, which was the k_{obs} value determined for the 0 mg C/L condition. While the presence of H_2O_2 may be related to rotavirus inactivation, the quenching experiments did not provide direct evidence that rotavirus inactivation was due to oxidation by H_2O_2. We therefore conducted another set of experiments, for which H_2O_2 was added at concentrations of 0, 6, 60, and 600 μM to rotavirus solutions kept in the dark at 50 °C. Up to 60 μM, these experiments did not show statistically different (p value range 0.17−0.58) inactivation kinetics ($k_{obs} \approx 0.45$ h^{-1}; or 1-log_{10} unit in 6 h). The condition with 600 μM H_2O_2 showed significantly greater rotavirus inactivation, with a k_{obs} value of 5.5 ± 3.3 h^{-1} (~1-log_{10} units in 0.5 h). Although the H_2O_2 formation rate by SRNOM irradiation increased with temperature (see Figure 3Sb), the maximum [H_2O_2] measured at 50 °C did not exceed 6 μM over the 3 h period of all rotavirus experiments at 50 °C. This suggests that H_2O_2 produced by UVA and visible light irradiation of solutions containing 20 mg C/L of SRNOM was not directly responsible for the inactivation of rotavirus.

DISCUSSION

Direct photolysis processes, such as those that cause direct damage to the genome by UVB light, are typically temperature-independent for noncellular systems lacking self-repairing mechanisms such as extracellular biomolecules and viruses.[52−54] In the absence of external sensitizers (SRNOM), the small slope of MS2 k_{obs} versus temperature (i.e., slope = 0.02 with $p = 0.0009$ for the temperature range of 14−42 °C) suggested that direct photolysis was responsible for MS2 inactivation by the full solar spectrum. For rotavirus irradiated with full spectrum sunlight at this same temperature range, the slope of k_{obs} versus temperature was greater (slope = 0.15; $p = 0.017$). This temperature dependence for rotavirus inactivation may stem from endogenous inactivation in the UVB range.[17,21] After the absorption of UVB light by the internal sensitizers such as RNA and proteins, the excited sensitizers may react via bimolecular reactions with virus constituents directly, or may react with dissolved oxygen to generate inactivating ROS.[19] Such internal bimolecular reactions are likely to be enhanced by temperature[52] and may therefore be responsible for the observed increase in rotavirus k_{obs} values from 14 to 42 °C in the absence of exogenous sensitizers. However, the insignificant kinetics difference ($p = 0.85$) between rotavirus k_{obs} values obtained from solutions with and without SRNOM versus temperature (14−42 °C) suggested that SRNOM-mediated exogenous inactivation did not contribute significantly to the overall inactivation caused by full spectrum irradiation. Thus, for full spectrum irradiation, direct photolysis played a dominant role for rotavirus inactivation in the presence or absence of SRNOM. A similar conclusion about the role of direct photolysis was reported for adenovirus and poliovirus.[28]

The contribution of SRNOM to MS2 inactivation by full spectrum or UVA and visible light was studied with solutions containing 20 mg C/L SRNOM in the temperature range of 14−42 °C. Under UVA and visible light only, exogenous inactivation from the addition of SRNOM constituted a majority of inactivation and increased temperature dependence of disinfection as compared to the reactor without SRNOM (slope difference of 0.03 from k_{obs} vs temperature plots). Under full spectrum irradiation in the same temperature range, the addition of SRNOM-mediated exogenous inactivation also increased k_{obs} values and temperature dependence (slope difference of 0.04 from k_{obs} vs temperature plots). To compare the contribution of exogenous effects between full spectrum and only UVA and visible light, estimated full spectrum k_{obs} values in SRNOM were obtained by adding the k_{obs} value for the SRNOM-mediated exogenous inactivation measured under UVA and visible light (Figure 3c) to the k_{obs} value obtained from full spectrum irradiation in the absence of SRNOM (Figure 3a) at a given temperature. The plots (Figure 3a) generated from the estimated k_{obs} and the measured values for full spectrum irradiation in 20 mg C/L against temperature (14−42 °C) were not statistically different from each other ($p = 0.18$). Agreement between observed and estimated k_{obs} suggests that for MS2, the SRNOM-mediated exogenous effects contributed similarly to inactivation and temperature dependence regardless of the magnitude of endogenous and direct inactivation in solution or the addition of UVB.

Formation of ROS, including $^1O_2$, ·OH, and H_2O_2 by SRNOM solution irradiated with UVA and visible light, may be responsible for virus inactivation. We first discuss the temperature dependence of $^1O_2$ concentration on MS2 inactivation because $^1O_2$ is the oxidant responsible for MS2 inactivation in humic acid solutions.[27] Concentrations of $^1O_2$ (Figure 3Sa) did not significantly differ from a slope of zero with temperature from 14 to 42 °C ($p = 0.11$). Statistically faster exogenous inactivation of MS2 thus cannot be explained by increased $^1O_2$ concentration,

but is likely due to an increased reaction rate between ROS formed exogenously with the viral particles. Additionally, the MS2 capsid could become more permeable to ROS at increased temperature as observed for the canine parvovirus capsid,[55] which would allow the penetration of $^1O_2$ and other ROS to damage the genome more readily.

As shown in the Results, formation of H_2O_2 by SRNOM irradiated with UVA and visible light was essential for rotavirus inactivation at 50 °C, but the presence of <60 μM of H_2O_2 in the dark was not enough to explain the observed inactivation of rotavirus. Because H_2O_2 is thought to form as a secondary product of sunlight-induced reactions, the formation of H_2O_2 in SRNOM solutions exposed to sunlight is indicative of the formation of other reactive species potentially responsible for inactivating rotavirus.[51] For example, H_2O_2 is photochemically produced by the reaction between superoxide anion and triplet-excited state NOM.[56,57] H_2O_2 is also a promoter of ·OH formation in the presence of trace metals via the Fenton and Fenton-like processes, which may contribute to virus inactivation.[29] A trace metal analysis found 1.33 μM of iron and 6 nM of copper in the SRNOM solutions containing 20 mg C/L. Fe(III) and Cu(II) concentrations of ~1 μM at neutral pH have been shown to cause virus inactivation by (photo)-Fenton-like processes at 20 °C.[29] Although iron and copper are likely to be in complexation with NOM, at pH levels of 7.6−8.1 for the inactivation experiments, reaction rates of these complexes with H_2O_2 should be slow.[58,59] Besides photo-Fenton reactions, ·OH can also be produced by triplet state NOM.[43,56] The increased concentration of ·OH measured as a function of temperature (23−50 °C) suggested faster reaction for ·OH formation (Figure S3). Thus, rotavirus inactivation is likely related to triplet state NOM and ·OH formation in UVA and visible light irradiated solutions containing SRNOM.

MS2 and rotavirus are biologically different, which is potentially the primary characteristic influencing their unique responses to solar disinfection. Whereas MS2 bacteriophage is composed of a single protein capsid with a positive-sense RNA strand,[60] rotavirus consists of three concentric and complex protein capsids with 11 double-stranded RNA segments.[30] The structures of the protein capsids and the genomes of these two viruses may be responsible for the observed difference in their reactivity with individual ROS at temperature range of 14−42 °C. Currently, work is being done to investigate the molecular mechanisms of rotavirus inactivation by sunlight irradiation.

Environmental Relevance. The data reported here showed that temperature plays an important role in the sunlight-mediated inactivation of rotavirus and MS2. As compared to indirect inactivation, direct UVB irradiation was indisputably the most effective for both viruses in the temperature range considered (14−42 °C), and it was also the least influenced by temperature. At ambient temperature of 20−25 °C, rotavirus inactivation by full spectrum irradiation was dominant. Similar results have been observed for adenovirus type 2 and poliovirus type 3.[28] SRNOM-mediated exogenous inactivation was temperature-dependent for both viruses. For rotavirus, SRNOM-mediated exogenous inactivation was observed to be significant only in the 33−50 °C temperature range.

The temperatures of surface water bodies measured in the upper layers (4−30 cm depths) have been shown to range from 0 to 35 °C depending on the season, with fluctuations of up to 14 °C between day and night.[41] The results from this study suggest that inactivation of rotavirus in surface water may change significantly depending on sunlight intensity, cloud coverage, and UV index. The top layer of surface water is also important for virus disinfection because attenuation of UV irradiation by high NOM concentrations and/or suspended solids is minimized at shallow depths (0−20 cm).[61] Because UVB irradiation is more rapidly attenuated by high NOM systems rather than UVA irradiation, UVB effects would only be significant at very short depths (0−10 cm), and the indirect exogenous effects would contribute to virus disinfection at lower depths, particularly at high temperatures (>33 °C).

Besides the temperature and NOM concentration governing sunlight inactivation, other factors such as pH, type and origin of NOM, the presence of suspended solids, and biological activity should be considered for predicting inactivation of rotavirus.

■ ASSOCIATED CONTENT

Supporting Information. Additional experimental details, figures, and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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