Influence of algal organic matter on MS2 bacteriophage inactivation by ultraviolet irradiation at 220 nm and 254 nm

Yulin Wang a, Elbashir Araud b, Joanna L. Shisler c, d, Thanh H. Nguyen b, c, **, Baoling Yuan a, *

a Institute of Municipal and Environmental Engineering, College of Civil Engineering, Huaqiao University, Xiamen, Fujian 361021, PR China
b Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, 205 N. Mathews Ave., Urbana, IL 61801, United States
c Institute of Genomic Biology, University of Illinois at Urbana-Champaign, 205 N. Mathews Ave., Urbana, IL 61801, United States
d Department of Microbiology, Institute of Genomic Biology, University of Illinois at Urbana-Champaign, 601 S. Goodwin, Urbana, IL 61801, United States

** Corresponding author. Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, 205 N. Mathews Ave., Urbana, IL 61801, United States.
E-mail addresses: thn@illinois.edu (T.H. Nguyen), yuanbl@hotmail.com, blyuan@hqu.edu.cn (B. Yuan).

ARTICLE INFO

Article history:
Received 26 April 2018
Received in revised form 6 September 2018
Accepted 12 September 2018
Available online 17 September 2018
Handling Editor: Xiangru Zhang

Keywords:
Disinfection
Algal organic matter
MS2 bacteriophage
UV irradiation

ABSTRACT

We determined the potential interference of extracellular algal organic matter (EAOM) and intracellular algal organic matter (IAOM) extracted from Microcystis aeruginosa on MS2 bacteriophage inactivation under UV irradiation at two wavelengths (220 and 254 nm). UV irradiation at 220 nm doubled the inactivation rate of MS2 in water containing EAOM than in organic-free phosphate buffered solution. In contrast, EAOM did not change MS2 inactivation by exposure to UV 254 nm. The presence of IAOM did not significantly influence MS2 inactivation by exposure to either UV 254 or UV 220 nm. To achieve 3 log10 inactivation of MS2, UV254 nm required more than double the dose of UV220 nm (45 mJ/cm2 vs. 20 mJ/cm2). Linear correlations between the reduction in infectivity and the reduction in genome copies detected by reverse transcription quantitative polymerase chain reaction suggested that genomic damage is the main mechanism responsible for MS2 inactivation in water containing algal organic matter (AOM) by exposure to UV irradiation. These findings suggest that the presence of AOM did not negatively influence MS2 inactivation by either 220 or 254 nm irradiation, and that a lower UV dose of 220 nm irradiation can be used to achieve the same level of inactivation in water containing AOM.

© 2018 Elsevier Ltd. All rights reserved.

1. Introduction

Eutrophication in surface water (e.g., reservoirs, lakes, and rivers) can cause algal blooms. Extracellular algal organic matter (EAOM) is released into water during algae growth, while intracellular algal organic matter (IAOM) contaminates water during algal cell lysis. The negative effects of algal organic matter (AOM) on drinking water quality and treatment processes include an increased disinfection byproduct formation (Goslan et al., 2017), additional oxidation (Deng et al., 2017), and severe membrane fouling (Xu et al., 2018). Microcystis aeruginosa is used as the model blue-green algae to study the effect of organic matter produced by algae, including EAOM and Eammon drinking water treatment processes (Xu et al., 2018). UV disinfection is an important technology for water treatment because of its effectiveness in inactivating a wide range of pathogens (Hijnen et al., 2006). However, it is not known whether the presence of Microcystis aeruginosa exudates in the source water influences the efficacy of UV irradiation-
based disinfection. Algal exudates may decrease light penetration and reduce disinfection efficacy, similar to the effects of reduction of UV disinfection efficacy for drinking water with higher turbidity (Cantwell and Hofmann, 2011). The formation of reactive radicals from the exudates under irradiation may enhance disinfection efficacy (Zhang et al., 2015, 2016; Garg et al., 2017). As eutrophication is a widespread problem threatening the safety of drinking water globally (Paerl et al., 2011; Sharpley and Wang, 2014), it is essential that the influence of AOM on disinfection efficacy be understood.

Viruses are present in surface water and groundwater, which are used for drinking water sources (Aw and Gin, 2010, 2011; Corsi et al., 2014; Kiulia et al., 2015; Fout et al., 2017). Due to the nanometer size of the viruses and the ionic interaction between viruses and the surfaces of membrane or granular filtration (Lu et al., 2017), virus removal by conventional coagulation, granular filtration, or membrane filtration is often unreliable (Mi et al., 2005). Because waterborne viruses are a public health concern (Gall et al., 2015), the EPA has proposed to regulate and control virus contamination levels for safe drinking water (USEPA, 2016). UV irradiation effectively inactivates waterborne viruses (Hijnen et al., 2006; Linden et al., 2007; Brownell et al., 2008; Feng et al., 2016; Wu et al., 2018). UVC irradiation induces both genomic and protein capsid damage to inactivate bacteriophages (Brown et al., 2009; Beck et al., 2014, 2018; Vazquez-Bravo et al., 2018). Many laboratories around the world conduct experiments with bacteriophage MS2, which is a single-stranded RNA virus similar to norovirus, the most common cause of acute gastroenteritis (Hall et al., 2014; Moore et al., 2015). The correction factors for the average photon irradiance at 220 nm or 254 nm. These findings can be used as a surrogate for RNA viruses are valuable because they allow a comparison among different disinfection technologies, from conventional to emerging (Brownell et al., 2008; Lee et al., 2011; Zhuo et al., 2015; Amarasi et al., 2017; Rattanakul and Oguma, 2017; Park et al., 2018).

The objective of this study was to fill the knowledge gap on how AOM influences the inactivation efficacy of MS2 by UV irradiation at two wavelengths (220 and 254 nm). These two wavelengths were selected because 254 nm is commonly used in drinking water disinfection and 220 nm has been found to be more effective for MS2 and adenovirus inactivation than the longer wavelength (Beck et al., 2016; Vazquez-Bravo et al., 2018). In addition, we also determined how the presence of AOM influences genomic damage of MS2 irradiated by 220 nm or 254 nm. These findings can be used by water or wastewater treatment facilities to design effective disinfection treatment for water laden with AOM.

2. Materials and methods

2.1. Preparation and characterization of Microcystis aeruginosa exudates

Microcystis aeruginosa (FACHB-905) was purchased from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences, China. Microcystis aeruginosa stock was prepared following a previous study (Fang et al., 2010). Briefly, Microcystis aeruginosa cells were cultured in sterilized BG11 media in a light incubator (CPJ-1500) at 25 ± 1°C with an illumination of 2000 lux for 16 hrs daily for three months. The culture was shaken twice a day during the incubation period.

The AOM was produced from the Microcystis aeruginosa suspension grown until post-stationary phase. Algal cells solution was centrifuged at ~10,000 × g for 10 min to separate the algal cells from the suspensions. The EAOM was isolated by filtering the supernatant through cellulose acetate membranes with 0.45 μm pore sizes to remove the cells remaining in the suspension after centrifugation. The IAOM was produced from the algal cells by first subjecting cells to washing for three times, then resuspending in deionized water, and then freezing and thawing for three times. Lysed cells were centrifuged to remove cellular debris, and supernatants were filtered as for EAOM. These EAOM and IAOM solutions were diluted for the virus inactivation experiments, as described below.

The total organic carbon (TOC) concentrations of EAOM, IAOM, and surface water samples collected from the Jialong River and reservoirs, China, were quantified by using Shimadzu TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD). For disinfection experiments, the EAOM and IAOM solutions were diluted in organic-free phosphate buffered solution (PBS) at pH 7 so that the TOC concentrations of these solutions were 11.91 ± 0.56 mg/L, which was close to the values measured for surface water samples (11.49 ± 0.27 mg/L). In addition, three-dimensional excitation-emission-matrix (EEM) fluorescence spectrum analysis was performed on each sample by using a Shimadzu RF-5301PC spectrofluorometer, as described previously (Massalha et al., 2018). Samples were added to a quartz cuvette with 4 × 10 cm windows. Excitation wavelengths varied from 220 to 500 nm with increments of 10 nm, and emission wavelengths varied between 220 and 550 nm with 1 nm increments.

2.2. MS2 coliphage propagation and enumeration

MS2 bacteriophage (ATCC 15597-B1) was propagated and purified as described previously (Rosado-Lausell et al., 2013). Briefly, Escherichia coli (ATCC 15597) was grown in tryptic soy broth solution for 6 hrs and inoculated with 10^12 plaque forming units (PFU/mL) of MS2 that had been purified by sequential centrifugation at 1,500 × g for 10 min at 20°C (Sorvall Legend RT plus, Thermo scientific) and filtration through 0.22 μm membranes. The virus contained in the filtrate was further purified by ultracentrifugation (Beckman coulter optima XPN-90) at 82,000 × g through a 40% (wt/vol) sucrose cushion at 4°C for 3 hrs, in a Ty50.2 rotor (Beckman Coulter, Fullerton, CA). The MS2 pellet was re-suspended in 1 mL of PBS on ice overnight. The final MS2 stock at concentration of approximately 10^15 PFU/mL was divided into small aliquots and stored at −80°C. Enumeration of MS2 with the double agar layer method and M. coli bacterial host followed the method described previously (Adams, 1959; Rosado-Lausell et al., 2013).

2.3. UV disinfection experiments

A 1-kW Rayoex collimated UV system equipped with a polychromatic medium pressure (MPUV) lamp (A300014, Calgon Carbon Co., Pittsburgh, PA) was used for UV disinfection experiments. Bandpass optical filters with a full width at half maximum (FWHM) bandwidth of about 10 nm, and at half of the maximum peak transmission at 220 and 254 nm (Andover Corporation, Salem, NH) were placed at the end of the collimator to isolate irradiation at each of these wavelengths. The spectral irradiance of the light passing through the filter was measured by a BLK-C-50 spectrometer (Stellar Net Inc. Tampa, FL) to ensure the irradiation at wave-length of 220 nm or 254 nm. The UV dose was determined by the method of Bolton et al. (2015) for a polychromatic source using the measured irradiance spectra obtained after the bandpass filters. We first did the calculation for the average photon irradiance rate in the water with the unit of Einstein m^-2 s^-1 nm^-1, then we converted this average photon irradiance rate to the equivalent average irradiance at 254 nm in the water with unit ml/cm^2. The latter is referred to as the UV dose to be consistent with other previous studies (Bolton and Linden, 2003; Beck et al., 2014, 2016; Bolton et al., 2015). The correction factors for the average photon irradiance rate in water include the Petri factor, reflection factor, water
factor and divergence factor (Bolton and Linden, 2003; Bolton et al., 2015). To calculate the Petri factor, the UV irradiance was measured by a BLK-C-50 spectrometer (StellarNet Inc. Tampa, FL) at every 0.5 cm in the x and y directions over the area of the reactor of the spectral irradiance of the light passing through the filter. The water factor is calculated based on the UV absorbance spectra of the solutions used for the disinfection experiments and were measured with a UV-2700 Shimadzu UV spectrophotometer. The irradiance through the bandpass filter was measured with a 1400A radiometer and a SEL 240 detector to ensure that the emission through the filter was around the wavelength of 220 nm or 254 nm (Fig. 1S). The average photon fluence rate and the UV dose were not corrected for the germicidal factors because of the comparable results at a given UV dose by this study in PBS and by Beck et al. (2016), who also did not make this correction. For MS2 inactivation, 50 mL Pyrex beakers with 21 mL of a water sample and an initial MS2 concentration of 10^{10} PFU/mL were used. During UV irradiation, the solution was continuously stirred by a magnetic stir bar set at 300 rpm. One mL samples were collected at the beginning of the experiment and at different sampling times. Each sample was collected in a 1.7 mL autoclaved centrifuge tube and stored at −80 °C for both plate assays and qPCR. All Pyrex beakers were wrapped with black tape to prevent light scattering. For the dark control experiments, the reactors were covered completely with foil. For each condition, at least three replicates were conducted. Inactivation data were presented as either natural log (Ln) or log_{10} of C/C_{0}, where C was infectivity determined at time t and C_{0} was infectivity of the sample taken at time zero before the solution was irradiated. C/C_{0} is referred to as reduction in infectivity.

2.4. Measurement of radical concentrations

To compare the concentrations of hydroxyl and singlet oxygen radical concentrations produced under each experimental condition, phenol and furfuryl alcohol (FFA) were used as probe compounds, respectively, as described previously (Haag and Hoigne, 1986; Kochany and Bolton, 1991). Each probe compound was added to different water samples at a final concentration of 100 μM. These solutions were exposed to UV radiation while being continuously stirred by a magnetic stir bar set at 300 rpm. To measure radical production under dark conditions, as a control, the reactors were completely covered with foil. Samples were taken at the beginning of the experiment and throughout the experiment. Collected samples were kept in 2 mL amber vials with screw caps and PTFE seals at 4 °C before HPLC analysis. All of the samples were analyzed within 4 hrs. Phenol concentrations were determined using a reverse-phase high performance liquid chromatography (HPLC) (Agilent technologies, USA) and a ZORBAX Eclipse Plus C18 column (2.1 mm × 100 mm internal diameter 3.5 μm). The mobile phase of acetic acid and nanopure water (45:55, v/v) was used at a flow rate of 0.3 mL/min and column temperature at 20 °C. Absorbance measurement was recorded at 268 nm. The retention time found with the results with the phenol standards was 2.5 ± 0.1 min. FFA concentrations were analyzed by the same HPLC system but with a ZORBAX Eclipse XDB C18 column (4.6 mm × 150 mm internal diameter 5 μm). For FFA, the mobile phase of 35% methyl alcohol and 65% of 0.1% acetic acid by volume at a flow rate of 0.5 mL/min was used. Absorbance measurement for FFA was recorded at 216 nm. The retention time found from the results with the FFA was 6.0 ± 0.1 min. The concentrations of hydroxyl radicals and singlet oxygen radicals were determined by multiplying the pseudo-first-order decay constant of the probe chemicals by the corresponding quenching rates, which are 1.4 × 10^{20} M/s and 1.2 × 10^{18} M/s, respectively (Haag and Hoigne, 1986; Kochany and Bolton, 1991). For each condition, three replicates were conducted for radical measurement.

2.5. Detection of viral genome damage

Viral RNA was extracted from each sample by using an RNA extraction kit (QIAamp Viral RNA Mini kit), following the manufacturer’s instructions. The extracted RNA sample was stored at −80 °C until being subjected to reverse transcription quantitative polymerase chain reaction (RT-qPCR). A two-step RT-qPCR was used to quantify MS2 genome damage, following a method developed previously (Beck et al., 2016). Briefly, two reverse primer sets targeting genomic regions 3405–3424 or 2418–2440 were used to reverse transcribe RNA into cDNA of 2169 bp or 1185 bp, respectively. Specifically, for the reverse transcription step, 10 μL of the RNA sample and 2 μL of either reverse primer 3424 (25 μM) (5′-TCT TTC GAG CAC ACC CAC C-3′) or reverse primer 2440 (25 μM) (5′-TCT ATA CCA ACG CAT GTG AGC C-3′) were mixed together and incubated at 70 °C for 5 min, and then rapidly chilled on ice for 1 min. cDNA was synthesized when M-MulV Reverse Transcriptase was added to each reaction, following manufacturer’s directions. Reactions were heated at 42 °C for 1 h and then chilled on ice. Next, 3 μL of cDNA-containing solution was added to a 7 μL solution containing 2 μL Taq universal SYBR green reaction mix, 0.125 μL of iScript reverse transcriptase, 0.3 μL of 25 μM reverse primer (5′-ACC CCT TGA AAG AGT CTG C-3′), 0.3 μL of 25 μM forward primer (5′-TCG ATG GTC CAT ACC TTA CAT GC-3′) and 1.25 μL of nuclease-free water. The thermal cycling conditions for qPCR were: 94 °C for 2 min to activate the transcriptase enzyme, 40 cycles of DNA denaturing at 94 °C for 15 s, and annealing and extension at 60 °C for 30 s. This two-step RT-qPCR allowed quantification of fragments longer than typical one-step RT-qPCR because cDNA was produced only on the fragments of 2169 or 1185 bp that are not damaged. The second step of qPCR allowed the quantification of the cDNA by using a primer pair targeting genome positions 1255–1277 (forward) and 1404–1423, to amplify an amplicon of 168 bp following a previous study (Beck et al., 2016).

The RT-qPCR standard curves were established using 10-fold serial dilutions of solution with known concentrations of MS2 cDNA. The following calibration curves were found:

Log_{10} copies of 1185 bp fragments = 13.409−0.2806 (CT) R^{2} = 0.99, Efficiency = 91%

Log_{10} copies of 2169 bp fragments = 13.498−0.2749 (CT) R^{2} = 0.99, Efficiency = 90%

These two standard calibration curves then were used to determine the copy numbers of the two MS2-based amplicons from samples collected at different times during the irradiation experiments. The data were presented as either log_{10} or ln of (N/N_{0}), where N is the copy number measured for a sample collected at time t and N_{0} is the copy number measured for a sample collected right before the solution was irradiated. N/N_{0} is referred to as the reduction in copy number. Potential PCR inhibition was avoided by sequential dilution of the samples.

2.6. Statistical analysis

We used all collected data above the detection limit for linear regression analysis. The following linear regressions were conducted: log_{10} reduction in infectivity as a function of UV dose. In reduction in infectivity as a function of ln reduction in copy number, and log_{10} reduction in copy number as a function of UV dose. Coefficient of determination (R^{2}) and Pearson’s coefficient were calculated for each linear regression to assess the goodness of fit.
and the correlation strength, respectively. ANCOVA with significance level of 95% was used to compare two regression lines with indicator variables of 0 for the control (e.g., PBS solution) and 1 for the EAOM or IAOM solution. In addition, two-way ANOVA with UV dose as one factor and solution as another factor was conducted for results obtained for 254 nm irradiation experiments.

3. Results and discussion

3.1. Formation of hydroxyl and singlet oxygen radicals by EAOM or IAOM solutions irradiated at 220 nm or 254 nm wavelength

We first characterized the UV absorbance and fluorescence of the EAOM and IAOM solutions because their presence may influence the efficacy of MS2 inactivation by 220 nm or 254 nm irradiation. The specific UV absorbance (SUVA) values of EAOM and IAOM were measured at 1.18 ± (0.01) and 2.09 ± (0.04), respectively. The higher SUVA of IAOM compared to EAOM suggested that IAOM has higher aromacity than EAOM. The fluorescence of EAOM and IAOM are shown in Fig. 2S. EAOM had the highest intense peak (137 AU/mg C/L) at Excitation/Emission (Em/Ex) of 437/250 nm, which was within the range of the fulvic acid-like region, as proposed previously (Chen et al., 2003). The second highest intense peak (107 AU/mg C/L) at Em/Ex of 427/340 nm was also in the humic acid-like region. The third highest intense peak (100 AU/mg C/L) appeared at Em/Ex of 339/330 nm, which was in the region for soluble microbial product.

IAOM had the highest intense peak, (1824 AU/mg C/L) at Em/Ex of 480/240 nm in the fulvic acid-like region. The second peak was observed at 426 AU/mg C/L at Em/Ex of 453/410 nm in the humic acid-like region (Table S1). The UV absorbance and fluorescence spectra of EAOM and IAOM suggest that these organic matters can absorb UV irradiation to produce exogenous reactive radicals, which can influence MS2 inactivation, as recently reviewed (Nelson et al., 2018).

We also compared the concentrations of hydroxyl and singlet oxygen radicals formed by AOM due to irradiation at 220 nm or 254 nm. Using phenol and FFA as probe chemicals of hydroxyl and singlet oxygen radicals, respectively, we observed that under UV220-irradiation at 1.74 mJ/cm², EAOM-containing water respectively produced hydroxyl radicals and singlet oxygen at concentrations of (9.25 ± 1.55) × 10⁻¹⁵ M and (727 ± 314) × 10⁻¹² M (Table S2). The decay rates of the probe compounds receiving 220 nm irradiation were significantly higher than those samples that were incubated in the dark (P = 2 × 10⁻⁹ for hydroxyl and P = 9 × 10⁻⁴ for singlet oxygen, Table S3), suggesting the production of radicals by UV irradiation. In contrast, the degradation rates of the probe compounds receiving 254 nm irradiation (at 38.71 mJ/cm² in EAOM solution and at 32.93 mJ/cm² in IAOM solution) were not significantly different from those obtained from samples incubated in the dark (P = 0.11 for phenol and P = 0.86 for FFA, Table S3). In addition, degradation rates of these probe compounds in IAOM by irradiation at UV 254 nm or UV 220 nm were not significantly different from samples incubated in the dark (P > 0.05, Table S3). Thus, the hydroxyl and singlet oxygen radicals were not produced at high enough concentrations to be detected in the EAOM solution irradiated by 254 nm or in the IAOM solution irradiated by either 220 nm or 254 nm.

3.2. The effect of algal organic matter on MS2 coliphage inactivation by UV irradiation

The influence of AOM on MS2 inactivation by UVC irradiation was studied by analyzing the MS2 inactivation in the presence or absence of AOM. As shown in Fig. 1 (a), EAOM or IAOM did not influence MS2 inactivation rates when solutions were irradiated with 254 nm. The P-values were greater than 0.05 for either ANOVA or pair-wise comparison amongst the three solutions (EAOM, IAOM, and organic-free PBS, Table S4). An approximate 3 log₁₀ reduction in MS2 infectivity was observed for each solution after 254 nm irradiation at a dose of approximately 40 mJ/cm². Similarly, the presence of IAOM did not significantly alter MS2 inactivation by 220 nm irradiation (P = 0.14, Table S4). At a UV dose of 25 mJ/cm² of 220 nm irradiation, there was a reduction in MS2 infectivity in the PBS and in the IAOM solutions (3.1–5.2 log₁₀ and 3.3–4.0 log₁₀, respectively). In contrast, the presence of EAOM significantly increased MS2 inactivation at all tested UV doses of 220 nm irradiation (P = 7 × 10⁻⁶, Fig. 1 (b), Table S4). A 5.4 to 6.2 log₁₀ reduction in infectivity for 19 mJ/cm² was observed in the EAOM solution irradiated with 220 nm. We also observed that 220 nm irradiation significantly increased MS2 inactivation in all tested UV doses of 220 nm irradiation (P = 3 × 10⁻² for PBS, P = 5 × 10⁻¹⁴ for IAOM and 5 × 10⁻¹⁰ for EAOM, Table S8). To achieve up to 3 log₁₀ of MS2 inactivation in each of the solutions examined here, close to 40 mJ/cm² UV dose was required for 254 nm, while 12.6 mJ/cm² (for EAOM) or 19 mJ/cm² (for PBS or IAOM) was needed at 220 nm. Higher potency of 220 nm irradiation compared to 254 nm irradiation has also been reported by previous studies for MS2 inactivation in solutions without organic matter (Rodriguez et al., 2014; Beck et al., 2016).

3.3. Detection of MS2 genome damage from irradiation with UV at 220 nm or 254 nm

Genome damage of MS2 in water containing AOM irradiated by
220 nm or 254 nm was detected by RT-qPCR targeting two MS2 fragments of 1185 bp and 2169 bp. We found linear correlations between the reduction in copy numbers of the amplicons obtained from MS2 after irradiation and the corresponding UV doses (Fig. 2 (a) and (b)). As shown in Fig. 2 (a), UV 254 nm irradiation resulted in up to 1 log10 reduction of the copy number of these two MS2 amplicons at UV dose of approximately 40 mJ/cm². Note that although these two amplicons have different lengths (1185 bp and 2169 bp), a linear correlation with slope of 0.02 fit all data obtained for all three studied solutions (PBS, IAOM, and EAOM). This correlation has R² value of 0.96 and Pearson’s coefficient value of 0.98 (Table S6). These high values of R² and Pearson’s coefficient indicate the goodness of fit for the linear correlation. Beck et al. (2016) found a slope of 0.046 for 1185 base pair and 0.058 for 2169 base pair for MS2 inactivation in PBS. These slopes were two and three times higher, respectively, than the slope of 0.02 found in our study. This discrepancy may be due to several factors, including RT-qPCR efficiencies. Nevertheless, our data and data from Beck et al. (2016) show evidence of genome damage causing MS2 inactivation by UV 254 nm irradiation. In addition, compared to the PBS solution, the presence of either EAOM or IAOM did not influence the rate of genome damage caused by UV 254 nm. Previous studies have found that UV 254 nm irradiation did not cause MS2 protein damage or MS2 attachment to E. coli host cells (Beck et al., 2014; Rattanakul and Oguma, 2017). Based on the results of these previous studies and the similarity in results obtained for PBS, EAOM, and IAOM, it appears that irradiation at 254 nm of EAOM or IAOM solutions also did not lead to MS2 protein damage nor additional RNA damage. Insufficient concentrations of hydroxyl and singlet oxygen radicals produced in EAOM and IAOM solutions by 254 nm irradiation may explain the insignificant influence of EAOM and IAOM.

There was a 1.0 log10 reduction in MS2-based amplicons after either the PBS or IAOM solution was irradiated with 220 nm for 25 mJ/cm² (Fig. 2 (b)). Under irradiation by UV220 nm, the difference in the reduction of copy numbers obtained by targeting the longer fragments (2169 bp) or shorter fragments (1185 bp) in water containing EAOM or IAOM was not significant (P > 0.05, Table S5). Under irradiation by UV254 nm, the reduction of copy numbers obtained by targeting the two RNA fragments was shown to be insignificantly different in water containing EAOM and PBS (P > 0.05, Table S5). The slope of the regression line for MS2 samples in PBS or IAOM irradiated by 220 nm is 0.04, which is twice the slope of the regression line obtained for all samples after 254 nm irradiation. This higher slope for 220 nm-irradiated samples meant that, at a given UV dose, a higher reduction in copy numbers was observed with 220 nm-irradiated samples compared to 254 nm-irradiated samples. Note that Beck et al. (2016) also found higher rate constants for RNA damage in MS2 irradiated by 220 nm than those by 254 nm irradiation. A reduction of 2.3 log10 and 1.7 log10 MS2 genome copy numbers was observed for the EAOM solution being irradiated by 18.9 mJ/cm² irradiation at 220 nm, as detected by targeting the shorter region of 1185 bp and the longer region of 2169 bp, respectively (Fig. 2 (c)). In addition, the data obtained for MS2 in the EAOM solution irradiated with 220 nm were fitted with two linear regressions. With UV dose up to 15 mJ/cm², the regression has a slope of 0.03, which is in the range of the slopes found for 254 nm irradiation or 220 nm irradiation of PBS or IAOM solutions. However, from 15 mJ/cm² to 25 mJ/cm², the slope of the regression line is 0.22, indicating that at the higher UV dose, much faster reduction in the copy number of the targeted fragments was observed, compared to the lower UV dose.

To analyze the correlations between genome damage and inactivation, we plotted the reduction in PFU vs. the reduction in the copy numbers of MS2-based amplicons (Figs. 3 and 4). We used the natural log scale instead of log10 so that we can compare our analysis with a previous study (Pecson et al., 2011). According to Pecson et al. the ratio between the natural log of undamaged genomes (as quantified by PFU) and the natural log of undamaged amplicons (as quantified by qPCR) can be used to assess whether the lesion rate per nucleotide is constant across the viral genome. To determine the ratios of the natural log of undamaged genome to the natural log of undamaged amplicons, we determined the slopes of the regression lines of all MS2 collected from the irradiation experiments, as shown in Figs. 3 and 4. One linear regression with R² of 0.93 and Pearson’s coefficient of 0.90 was found for all MS2 data from 254 nm irradiation experiments using either PBS, EAOM, or IAOM (Figs. 3 (a) and 4 (a), Table S7). The slopes of the correlations formed with the 1185 bp fragment and the 2169 bp fragment are 3.5 and 2.7, respectively. If the lesion rate per nucleotide was indeed constant across the MS2 genome, then these slopes should be equal to the ratio of the total genome over the fragment length, i.e., 3.0 for 1185 bp and 1.7 for 2169 bp. Our results suggest that the lesion rates of both targeted MS2 genomic regions were higher than the lesion rates across the whole genome.

When the 1185 bp fragment was targeted, one regression line
with $R^2$ of 0.95 and Pearson’s coefficient of 0.94 can fit the data obtained for MS2 in PBS or IAOM solutions irradiated with 220 nm (Fig. 3 (b), Table S7). The slope of this line is 4.06, which is also greater than 3.0, as in the case when the lesion rate is constant across the MS2 genome. As shown in Fig. 4 (b), when we targeted the 2169 bp fragment, the slopes of regression lines obtained for MS2 in PBS or IAOM irradiated with 220 nm are 4.9 and 3.5, respectively, which are again higher than 1.7, indicating again that the lesion rates of these two fragments were higher than that across the whole genome. Nevertheless, results obtained when examining either genomic fragment showed that MS2 inactivation by irradiation of PBS or IAOM solutions was caused by genome damage, in agreement with results obtained for MS2 inactivation in PBS reported previously (Beck et al., 2016).

As shown above, 220 nm irradiation of EAOM solutions caused highest inactivation amongst all studied conditions. In contrast to the steady increase of reduction in PFU, the regression lines were needed to fit the data for MS2 in EAOM solutions irradiated by 220 nm. Similar slopes of 12.0 and 12.3 ($P > 0.05$) were observed for MS2 in EAOM irradiated by 220 nm at the UV dose up to about 12 mJ/cm$^2$ (Figs. 3 (c) and 4 (c)). Afterward, these slopes reduced to 0.7 and 0.85 for 1185 bp and 2169 bp fragments, respectively (Figs. 3 (c) and 4 (c)). This change in slope suggests that the lesion rates were very high at the first irradiation period by 220 nm, then these rates decreased over time. It is likely that the genome was damaged in multiple places as the UV dose increased. However, because the two-step RT-qPCR assay cannot distinguish between single versus multiple genome mutations, further damage of the genome at multiple points as UV increased was not quantified. In addition, the increased rapidity of MS2 genome damage caused by 220 nm irradiation in an EAOM-containing solution, as compared to all other irradiation conditions, may be a compound effect of direct UV and of reactive radical damage to the genome.
3.4. Mechanistic and practical considerations

Data above showed that even in conditions mimicking surface water highly polluted with AOM, 220 nm irradiation is more potent than 254 nm irradiation for MS2 bacteriophage inactivation. This is due to the fact that 220 nm irradiation has higher energy per photon than 254 nm irradiation has (5.6 eV vs. 4.9 eV). By examining close to 60% of the viral genome, we found clear evidence that MS2 inactivation was a direct result of genome damage caused by UV irradiation, and high levels of genome damage was observed for 220 nm compared to 254 nm irradiation. These findings agree with studies using adenosine virus (Linden et al., 2007; Eischeid et al., 2009; Beck et al., 2014; Corsi et al., 2014; Vazquez-Bravo et al., 2018), which has a double-stranded DNA genome. However, the lesion rates induced by UV irradiation to the ~35 kbp double-stranded DNA adenosine virus (Kennedy and Parks, 2009) versus the ~3.7 kbp single-stranded RNA MS2 genome are not likely to be similar. Thus, robust design of UV disinfection will require both data from laboratory experiments using enteric viruses with public health concerns and from pilot studies using MS2 as surrogates. If these two studies are conducted with the same water and the same irradiation conditions for genome damages in both viruses, the inactivation of adenovirus in the pilot setup may be estimated based on ratios of MS2 and adenovirus inactivation rate constants measured in the laboratory experiments.

EAOM is excreted from live cells, whereas IOM is released from lysed algal cells. Both components should be present in natural water polluted with excess nutrients. We found that hydroxyl radicals and singlet oxygen radicals were produced at detectable concentrations only by EAOM solution irradiated with 1.74 mJ/cm² of 220 nm. While we were not able to detect radical concentrations at the same UV dose as for the MS2 inactivation experiments, we assume that the EAOM solution irradiated by 220 nm produced relatively higher radicals than EAOM irradiated by 254 nm or IOM irradiated by 254 or 220 nm. This difference in radical formation seems to influence the lesion of the MS2 genome by UV irradiation. Specifically, in the EAOM solution irradiated by 220 nm, the log₁₀ reduction in copy numbers of the two genome fragments that are 33% and 60% of the whole MS2 genome increased at a slower rate at the UV dose up to 12 mJ/cm² and about 7 times higher at the UV dose from 12 mJ/cm² to about 20 mJ/cm². In all other cases, the log₁₀ reduction in copy numbers of these two genome fragments is linearly increased with the UV dose. Because reactions between reactive oxygen species (ROS) with RNA have been reported (Fimognari, 2015), we propose the following mechanisms. When ROS is produced at low concentrations, e.g., 254 nm irradiation of IOM or EAOM or 220 irradiation of IOM and low UV dose of 220 nm irradiation of EAOM, the lesion rate of MS2 RNA is a direct result of photons attacking the genome. When ROS is produced at high concentration, e.g., greater than 12 mJ/cm² of 220 nm irradiation of EAOM, in addition to RNA genome damage by photons, ROS can react with the MS2 protein capsids and subsequently MS2 genome. The combination of photons attacking and ROS reaction may cause enhanced RNA damage. It is outside the scope of this study to elucidate the role of ROS in viral genome damage and virus inactivation by UV irradiation.

4. Conclusions

UV irradiation is second only to chlorination for use as a common disinfection technology. Our findings here show, for the first time, that the presence of AOM did not negatively influence inactivation of MS2 bacteriophage by either UV 254 or 220 nm. EAOM enhanced inactivation when UV 220 nm was used. These findings suggest that UV irradiation can be used to disinfect drinking water even when this water source is contaminated with algal bloom.

Acknowledgements

The authors greatly appreciated the financial support from the Program for the Natural Science Foundation of China (Grant No. 51678255), the Key Projects of Enterprise and University Cooperation in Fujian (Grant No. 2018Y4010) and USEPA (Grant No. R835826). Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the USEPA. Further, the USEPA does not endorse the purchase of any commercial products or services mentioned in this publication. We acknowledge Prof. Marinas and Dr. Vazquez-Bravo for their guidance on the UV experimental setup.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2018.09.065.

References

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2018.09.065.

References

Gall, A.M., Marinas, B.J., Lu, Y., Shisler, J.L., 2015. Waterborne viruses: a barrier to...
safe drinking water. PloS Pathog. 11, 7.