Mechanism and efficacy of virus inactivation by a microplasma UV lamp generating monochromatic UV irradiation at 222 nm

Chamteut Oh\textsuperscript{a}, Peter P. Sun\textsuperscript{a,b}, Elbashir Araud\textsuperscript{a,c}, Thanh H. Nguyen\textsuperscript{a,d,*}

\textsuperscript{a}Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, United States
\textsuperscript{b}Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, United States
\textsuperscript{c}Holonyak Micro & Nanotechnology Lab, University of Illinois at Urbana-Champaign, United States
\textsuperscript{d}Institute of Genomic Biology, University of Illinois at Urbana-Champaign, United States

**A R T I C L E   I N F O**

Article history:
Received 19 May 2020
Revised 1 September 2020
Accepted 2 September 2020
Available online 4 September 2020

Keywords:
Microplasma UV lamp
Monochromatic UV irradiation at 222 nm
Adenovirus
Water disinfection
Molecular mechanisms

**A B S T R A C T**

This study evaluated the potential of a microplasma UV lamp as an alternative UV source to the current mercury-based (Hg-based) UV lamp for water disinfection. We developed a set of PCR-based molecular assays (long-range qPCR, DNase, and binding assay) to quantify the adenovirus genome, capsid, and fiber damage with a wide detection range (10^{15}-10^{6.5} PFU/mL). We used these molecular assays to characterize adenovirus (AdV) inactivation kinetics by microplasma UV that produced monochromatic UV at 222 nm. We found that the inactivation rate constant (0.142 cm\(^2\)/mJ) due to microplasma UV was 4.4 times higher than that of low-pressure Hg UV (0.032 cm\(^2\)/mJ). This high efficacy was attributed to monochromatic UV wavelength at 222 nm damaging the AdV capsid protein. The results of these molecular assays also proved that microplasma UV and medium-pressure Hg UV with a bandpass filter at 223 nm (MPUV\textsubscript{223nm}) have a similar influence on AdV (p<0.05). We then estimated the relative energy efficiency of MPUV and microplasma UV to LPUV for 4 log reduction of the viruses. We found that the microplasma UV resulted in higher inactivation rate constants for viruses than the current Hg-based UV. Consequently, microplasma UV could be more energy efficient than low-pressure Hg UV for water disinfection if the wall-plug efficiency of the microplasma UV lamp improved to 8.4% (currently 1.5%). Therefore, the microplasma UV lamp is a promising option for water disinfection.

© 2020 Elsevier Ltd. All rights reserved.

1. Introduction

Ultraviolet (UV) irradiation has been used increasingly for the disinfection of drinking water and wastewater treatment systems (Linden et al., 2019) because of its advantages over chemical disinfectants. In comparison to chlorination, UV irradiation does not produce harmful disinfection byproducts (DBPs) (Mitch et al., 2003), while it shows much higher disinfection efficiency to protozoa such as Cryptosporidium and Giardia (Hijnen et al., 2006). The compact size and wide spectrum of applicability of UV irradiation also allow it to be applied as a stand-alone disinfectant in small non-community systems where a residual disinfectant is not necessary (Dotson et al., 2012; Linden et al., 2019; Schalk et al., 2006). The majority of public water treatment facilities (97%) in the United States are classified as small systems (USEPA, 2016), and UV irradiation has been considered for microbial disinfection in these systems (Linden et al., 2019). UV irradiation has also been applied to a portable water purifier (Brownell et al., 2008). Therefore, the global market for UV disinfection equipment is expected to expand by $2.8 billion by 2020 (Song et al., 2016).

Low-pressure UV (LPUV) lamps widely used for UV disinfection in water treatment plants are filled with mercury (Hg) gas (about 10^{-5} bar) and use 40-500 W to generate monochromatic wavelength with a peak at 254 nm (Schalk et al., 2006). LPUVs are very effective for inactivating Cryptosporidium, Giardia, and pathogenic bacteria, while viruses are known to be less susceptible to this monochromatic wavelength (Chevrefils et al., 2006; Hijnen et al., 2006). Adenovirus (AdV) is particularly resistant to LPUV irradiation and requires high energy consumption for sufficient disinfection (Yates et al., 2006). The resistance of AdV to the LPUV irradiation can also increase the possibility of selective survival, potentially allowing the survival of viral species that are more resistant to UV irradiation at 254 nm (Rachmadi et al., 2018). Hg, the filling material for the LPUV lamp, is a hazardous chemical that may cause secondary contamination (Hou et al., 2012). In addition, the Hg-based UV lamps require about 30 minutes to warm up and stabilize the UV radiation. This time requirement is regarded as an
operational disadvantage (Song et al., 2016). A medium-pressure UV (MPUV) lamp has been proposed as a substitute for an LPUV lamp. This lamp is also filled with a higher pressure of Hg gas (1-6 bar) and uses 0.4-60 kW to generate polychromatic wavelengths ranging from 200 to 800 nm (Schalk et al., 2006). The polychromatic irradiation has a higher virus inactivation efficacy than the monochromatic irradiation at 254 nm (Chevreuil et al., 2006). However, a large portion of the input energy is used for generating irradiation outside the germicidal wavelength causing high temperature (up to 950°C) and low energy efficiency (Schalk et al., 2006). A UV light-emitting diode (LED) lamp was also recently discovered as a new UV source. The UV LED lamp has various advantages of diversity in wavelength, high energy efficiency, short warm-up time, Hg free materials, and durability (Song et al., 2016). However, UV-LED has low emission power and narrow wavelength in germicidal UVC (260-285 nm) (Würtz et al., 2011). Because of the different innate characteristics of UV lamps, a synergistic effect with the available UV lamps were studied (Hull and Linden, 2018; Beck et al., 2017). From this perspective, we first need to characterize emerging UV lamps with different UV wavelengths for their inactivation efficacies. This information is required to assess the potential application of these lamps for the disinfection of water and wastewater treatment. For example, Oguma et al. (2016) reported that the inactivation rate constant of monochromatic UV at 285 nm by UV LED for adenovirus type 5 was 15% higher than that by LPUV.

Microplasma UV irradiation is an emerging technology with several advantages over Hg-filled UV technology. The microplasma lamps have microcavities (< a few millimeters) filled with KrCl. Electrodes deliver time-varying voltages which interact with the gas to irradiate monochromatic UV with a peak at 222 nm (Park et al., 2019). Different wavelengths can also be produced depending on the filling gases (e.g., XeCl for 172 nm, XeCl for 308 nm). These microplasma UV lamps do not contain toxic substances such as Hg. Microplasma UV lamps can generate high power (115 mW) with small (25 cm²) and thin (<6 mm) lamp designs for different configurations of disinfection (Herring and Park, 2019). The short stabilization time (15 s) of the microplasma UV lamp allows intermittent disinfection, a higher energy efficiency, and a longer lifespan of the lamp (Raeiszadeh and Taghipour, 2019). These advantages of microplasma UV technology have the potential to overcome some shortcomings of the Hg-based UV lamps. Therefore, the microplasma UV lamp has been adopted in the medical field, in the semiconductor industry, and for air purification (Herring and Park, 2019; Welch et al., 2018). However, microplasma UV lamps have not been widely applied to inactivate viral pathogens for water and wastewater disinfection. This is probably because the unique geometry and spectral irradiance of the microplasma UV lamp have not been tested with viral pathogens. A study on the impact of the microplasma UV lamp on viral pathogens and comparison with commercially available UV lamps is needed.

The objective of this study was to characterize microplasma UV lamps as an alternative UV source for drinking water and wastewater disinfection. We conducted two analyses. First, we developed a set of molecular assays to quantify the damage in the genome, capsid protein, fiber protein, and infectivity of Adv by microplasma UV irradiation. Adv was selected because it is known as one of the most resistant waterborne viruses to the current Hg-based UV lamps (Chevreuil et al., 2006; Hijnen et al., 2006). The findings of these assays allowed us to understand why a microplasma UV lamp provided more efficient inactivation than the current Hg-based UV lamps. Second, we compared the energy efficiencies of different UV lamps. The higher rate constant for virus inactivation compared to the Hg-based UV lamps proposes the microplasma UV lamp as another viable option in designing a UV irradiation system. Therefore, the study findings will contribute to the potential application of microplasma UV lamps in water and wastewater treatment.

2. Materials and methods

2.1. Adv inactivation experiments by UV irradiation

2.1.1. UV irradiation by a microplasma UV lamp

A microplasma UV lamp (Eden Park Illumination, USA) had a 16 cm² effective illuminating area in a 5.08 × 5.08 cm (2 × 2 in) rectangular surface. The lamp was connected to an electric circuit, which was further connected to a wall plug. To ensure the stability of the power source, we used a special power supply designed by Eden Park Illumination. This power supply is capable of modulating input frequency, voltage, and current. A power meter was connected between the adaptor and wall plug to monitor the consumed total power and a ensure well-controlled power supply (Fig. 1). The spectral irradiance of the microplasma UV showed a monochromatic UV peak at 222 nm with a full width at half maximum of 3 nm (Fig. 1). The fluence applied to the Adv solution by microplasma UV was calculated by the following equations suggested by Raeiszadeh and Taghipour (2019).

\[ F = \int_{200}^{300} E \times PF \times \left( \frac{DF \times RF_s \times WF_s}{CF} \right) \times t \, d\lambda. \]  
\[ DF = \frac{1}{D_{FS}^{\alpha_s}} \left[ E(x) \right]_{\lambda}^{\lambda} \]  
\[ RF_s = 1 - \left[ \frac{n_{1,\lambda} - n_{2,\lambda}}{n_{1,\lambda} + n_{2,\lambda}} \right]^2 \]  
\[ WF_s = 1 - \frac{10^{-\alpha_s,\lambda}}{\alpha_s, \lambda} \]}

In these equations, F is the average fluence inside the Adv solution (mJ/cm²). E is the incident irradiance at the center of the solution surface (mW/cm²). PF was a petri factor, which was the ratio of the incident irradiance at the center to the average incident over the solution surface. The incident irradiance was measured by a UV power meter (C9536, Hamamatsu Photonics) with a UV power meter head (H9535, Hamamatsu Photonics), and PF was 0.906, 0.951, and 0.968 for 4, 5.5, and 7 cm distance between the lamp and the solution, respectively. DF was a divergence factor which was defined as the ratio of irradiance inside the empty volume to be filled with Adv solution during the experiment to the average irradiance at the solution surface. DF was calculated by Eq. (2) where D is the distance between the lamp and the solution surface, I is the solution depth, and E(x) is the UV irradiance at a certain distance. DFs (0.5 cm water depth) were 0.918, 0.929, and 0.939 for 4, 5.5, and 7 cm distance, respectively. RF was a reflection factor at which the UV reflection at the interface of two media with different reflective indices (n₁, λ and n₂, λ) was considered, and it is calculated using the Fresnel equation (Eq. (3)). CF was a collimation factor that was defined as the delivered irradiance to the fluence rate at the solution surface. The delivered irradiance can be calculated by a radiometry measurement, while the fluence rate at the solution surface can be determined by an actinometry experiment. The CF values were adopted from Raeiszadeh and Taghipour (2019), which measured it under the same experimental setup (i.e., microplasma UV lamp, petri dish size, and distance from the lamp) by the actinometry assay. The CF value for a 5.5 cm diameter dish with 4, 5.5, and 7 cm distance were 0.926, 0.972, and 0.996. WF was a water factor that addressed light attenuation in the solution. WF was defined as the ratio of average fluence inside the Adv solution to that at the solution surface (Eq. (4)). Here, \( \alpha_s(\text{cm}^{-1}) \) was the absorption coefficient of the solution at each wavelength, which was
measured using a spectrophotometer (UV-2450, Shimadzu, Japan). Finally, \( t \) was the exposure time (s). We conducted three virus inactivation experiments where the distance between the lamp and the solutions was 4, 5.5, and 7 cm. We found insignificant differences in the inactivation rate constants obtained with these three distances (\( p > 0.05 \) by Mann-Whitney test, Fig. S1). The four-centimeter distance was selected for the inactivation experiments using the microplasma UV lamp so that the viral solutions could be exposed to similar irradiance as that of the MPUV\(_{223\text{nm}}\) experiment. The UV irradiance was about 0.05 mW/cm\(^2\) at the center of the petri dish with a 4 cm distance from the lamp (Fig. 1).

The inactivation experiments were started with the preparation of 10.5 mL of the AdV solution in the quartz dish followed by taking the first sample (0.5 mL) before the lamp was turned on (i.e., fluence was 0 mJ/cm\(^2\)). Once the quartz dish with AdV solution (\( \sim 10^{4.5} \) PFU/mL) was exposed to UV irradiation, 0.5 mL of each sample was taken at certain time intervals for further analysis, including long-range qPCR, DNase, binding, and plaque assay. The changes in solution height were reflected in calculating the fluence by correcting WF and DF every sampling time (Table S1, S2, and S3). The UV irradiation experiment was replicated at least three times independently.

### 2.1.2. UV irradiation by an MPUV lamp with a bandpass filter

The MPUV lamp (Calgon Carbon Co., Pittsburgh, PA) was installed in a 1-kW Rayox collimated UV system for the UV irradiation experiments. Although the MPUV irradiated polychromatic UV wavelength ranged from 200 to 800 nm, the monochromatic UV wavelength was produced by a bandpass filter (Andover Corporation, Salem, NH) installed at the end of the collimator isolating wavelength of interest from the polychromatic wavelength. The monochromatic wavelength from the MPUV with the bandpass filter produced spectral irradiance with a peak wavelength at 223 nm and a full width at half maximum of 4 nm (Fig. 1). The MPUV with the bandpass filter will henceforth be referred to as the MPUV\(_{223\text{nm}}\). For AdV inactivation experiments, the AdV solution (\( \sim 10^{4.5} \) PFU/mL) was put in a quartz petri dish (5.5 cm diameter) and placed 50 cm away from the MPUV lamp. Similar to the microplasma UV experiment, 0.5 mL of each sample taken at certain time intervals was subjected to long-range qPCR, DNase, binding, and plaque assays. This distance provided the similar power of UV irradiance (0.05 mW/cm\(^2\)), which was applied to the microplasma UV experiment. The fluence inside the AdV solution was determined based on the previously suggested protocol (Bolton and Linden, 2003). The equation below was used to calculate the fluence. This equation was also used for the microplasma UV lamp, excluding CF because the collimator was assumed to ensure \( \text{CF}=1 \) (i.e., complete collimation). PF and DF were 0.852 and 0.992 in this study, respectively.

\[
F = \int_{200}^{300} E \times PF \times DF \times RF_{\lambda} \times WF_{\lambda} \times t \, d\lambda
\]

### 2.2. Molecular assays to reveal AdV inactivation efficacy and mechanisms

#### 2.2.1. Virus propagation and cell culture

The human lung carcinoma cell line, A549 (CCL-185), was obtained from American Type Culture Collection (ATCC, VA, USA). The cells were cultured in 175 cm\(^2\) flasks (Thermo Scientific\(^{\text{TM}}\), NH, USA) until the monolayer showed 80-100% confluency using Ham F-12 media with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, MA, USA) and 1X antibiotic-antimycotic (Thermo Fisher Scientific, MA, USA). These cells were either cultured for the new passage or used for further experiments, including virus propagation, binding assay, and plaque assay.

Human Adenovirus type 2 (VR-846) was also obtained from ATCC. The AdVs were propagated and purified for the inactivation experiment as follows. First, A549 cells with 80-100% confluency were prepared in a 175 cm\(^2\) flask. After removing the media, 2 mL of AdV solution was added to the flask and incubated at 37°C with 5% CO\(_2\) for 90 min to facilitate virus particle attachment to the host cells. After providing 20 mL of Ham F-12 media with 2% FBS and 1X antibiotic-antimycotic, the flask was incubated for 5 days for the viruses to make progenies. The viruses were separated from the A549 cells by three cycles of freezing and thawing. The cell debris was removed by centrifugation at 2000 rpm (556 g) for 10 min (Sorvall Legend RT Plus, Thermo Fisher Scientific, MA, USA), followed by filtration through a 0.45 μm filter (Millipore)
Sigma, MA, USA). The viruses were further separated from impurities such as proteins and nutrients using ultracentrifuge (Optima XPN-90 Ultracentrifuge, Beckman Coulter, CA, USA). A mix of 50 mL of the virus solution and 10 mL of the 40% sucrose solution (Thermo Fisher Scientific, MA, USA) was placed in a sterilized ultracentrifuge tube. The ultracentrifuge was then run at 10000 rpm (116 g) at 4°C for 5 min followed by 36000 rpm (150700 g) at 4°C for 3 hours. The supernatant was removed, and the virus pellet on the bottom of the ultracentrifuge tube was resuspended in 1X PBS (Thermo Fisher Scientific, MA, USA) and stored in -80°C until use.

2.2.2. Long-range qPCR assay to determine genomic damage

It is widely known that UV irradiation induces non-specific DNA lesions (Errol et al., 2006), so the longer amplicon size is preferred for identifying the UV-induced DNA damage (Rodriguez et al., 2013). However, the amplicon size that conventional qPCR can measure is less than 150 bp, which is too short compared to the AdV genome size (about 36 kb). Thus, we developed a long-range qPCR assay consisting of two sequential PCR steps where long-range amplicons (10 kbp) were synthesized in the first step, and then short-range amplicons (115 bp) were replicated from the first PCR product and quantified by qPCR in the second step.

DNA was extracted from AdV before and after exposure to UV irradiation using QIAamp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer’s protocol. The first PCR step for the long-range amplicon synthesis was conducted with 2 μL of the extracted DNA and 23 μL of PCR mixture from the LongAmp® Taq PCR Kit (New England Biolabs, MA, USA). The PCR cycle consisted of initial denaturation at 94°C for 30 s; 15 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 60 s, and extension at 65°C for 10 min; and the final extension at 65°C for 10 min. The first PCR product had mainly long-range (10 kbp) amplicons with a minor amount of single-stranded DNA and primers. The synthesis of long-range amplicons was confirmed by gel electrophoresis after 30 cycles of the first PCR step (Fig. S2).

In the second step, qPCR was used for the quantification of long-range amplicons from the first PCR product. Since the incomplete amplicons, ssDNA, and the remaining primers in the first PCR product would affect the second qPCR step, the impurities were removed by S1 nuclease (Thermo Scientific™, NH, USA) and subsequent QIAquick PCR Purification Kit (Qiagen, Germany). A mix of 5 μL of the first PCR product, 0.1 μL of S1 Nuclease, 6 μL of 5X Reaction Buffer, and 18.9 μL of nuclease-free water was incubated at 37°C for 30 min. Immediately after the S1 Nuclease reaction for the ssDNA degradation was completed, the PCR purification kit was applied to the mixture following the manufacturer’s protocol to remove the primers and obtain 50 μL of the purified PCR product. The long-range dsDNA was quantified by qPCR using a pair of primers targeting a 115 bp fragment on the 10 kbp long-range dsDNA. A mix of 2 μL of the purified PCR product, 5.0 μL of PowerUp SYBR™ Green Master Mix (Applied Biosystems, CA, USA), 0.3 μL of the reverse primer, 0.3 μL of the forward primer, and 2.4 μL of molecular biology grade water (Corning, NY, USA) was prepared. The qPCR step was run under the following conditions: initial denaturation at 95°C for 10 min; 40 cycles of denaturation at 95°C for 15 s, annealing and extension at 60°C for 1 min; and temperature increase for melting curve from 65°C to 95°C. An oligonucleotide (360 bp) covering the qPCR amplicon (115 bp) was designed (IDT Corporation, NJ), and the copy number was measured using a Qubit fluorometer (Invitrogen, CA, USA). For the absolute quantification of the PCR product, every qPCR was run with 10-fold serial dilutions of the known concentration of the oligonucleotide. The qPCR result was represented as an average value of at least three technical replications, and PCR efficiency was higher than 85% throughout this research. Detailed information about the primers and amplicons are presented in Table S4.

2.2.3. DNase assay to determine viral capsid damage

We developed a deoxyribonuclease (DNase) assay to check the AdV capsid integrity. It was assumed that DNase could not penetrate an intact viral capsid, but once the capsid is damaged, DNase can enter the virus and degrade its genomic DNA. This assay included a DNase reaction for DNA degradation of capsid-damaged virions and a subsequent long-range qPCR for quantifying the DNA degradation by DNase activity. First, the 50 μL virus samples were exposed to 2 μL of DNase with 10 μL of 10X DNase Buffer and 38 μL of RNase-free water (DNase Max kit, QIAGEN, Germany). After the mixture was incubated at 37°C for 40 min, 10 μL of DNase removal resin was added to the mixture and incubated at room temperature for 10 min with regular resuspension for homogenization. The mixture was centrifuged at 13300 rpm (17000 g) for 1 min to separate the resin from the solution. Seventy microliters of DNase-treated virus solution were taken, and the genome integrity was quantified by the long-range qPCR described in Section 2.2.2. Capsid damage was then determined based on the reduction in DNA copy number caused by DNase activity.

2.2.4. Binding assay to determine viral fiber damage

We conducted a binding assay that was slightly modified from Vazquez-Bravo et al. (2018) to evaluate AdV fiber integrity, which is an essential protein to bind to receptor proteins (e.g., coxsackievirus and adenovirus receptor) on the host cell membrane. For this assay, A549 cells were used as host cells. The A549 cell monolayers were prepared on 6-well plates (USA Scientific, FL, USA) with 80–100% confluency. The cells were inoculated with 200 μL of the virus solution and then incubated at room temperature for 90 min. The plates were gently shaken every 15 min to facilitate the virus particle attachment. Then, each well was washed three times with 1 mL of 1X PBS to remove unbound virions. The virus particles bound to cells were dislodged using 200 μL of 0.05% trypsin-EDTA (Thermo Fisher Scientific, MA, USA), and the trypsin activity was quenched using 1 mL of Ham’s F12-K media with 10% FBS. The solution was centrifuged at 13300 rpm (17000 g) for 10 min. The supernatant was removed, and the virus particles bound to cells were resuspended in 200 μL 1X PBS. Finally, the virus particles bound to the host cells were quantified by qPCR (Table S4) after DNA extraction. The number of housekeeping genes (β-actin) in A549 cells was also measured by qPCR to check if the ratio of host cells to viruses in this experiment impacts the binding assay result. Thermal cycling conditions for the housekeeping gene quantification were the same as the AdV qPCR (1 cycle of 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min). Detailed information for primers and qPCR conditions were presented in Table S5 and Section 2.2.2.

2.2.5. Plaque assay to determine viral infectivity

We developed a plaque assay to determine AdV infectivity. A549 cell monolayers were prepared on 6-well plates with 80–100% confluency. The virus solutions to be assayed were serially diluted with Ham’s F12-K media without FBS. The media in each well was aspirated, and 400 μL of each AdV dilution was inoculated into the cells. The plates containing the cells and viruses were incubated at 37°C and 5% CO2 for 90 min. The plates were gently shaken every 15 min to facilitate the virus particle attachment. The solution in each well was aspirated again, and the cells with viruses were covered by 2 mL overlay solution which was prepared by mixing 1.31 mL of 2X minimum essential medium (Thermo Fisher Scientific, MA, USA), 0.01 mL of 100X antibiotic-antimycotic, 0.05 mL of 15 mM HEPES, 0.03 mL of 7.5% sodium bicarbonate, 0.5 mL of 1% agarose solution, and 0.1 mL of FBS. After solidifying the overlay solution at 4°C for about 20 min, the plates were incubated at 37°C with 5% CO2 for 5 days. Two milliliters of the 10% formaldehyde solution in PBS was put into each well and
left at room temperature for two hours to fix the cells on the plate surface. After removing the overlay, the plaques were stained with 0.05% crystal violet in 10% ethanol for visualization (Fig. S3).

2.2.6. Reliability confirmation for the molecular assays

We confirmed the reliability of the three molecular assays (long-range qPCR, DNase, and binding) and plaque assay by conducting calibration curves to show the relationship between virus titer and the outcome from each assay. For calibration curves, 10-fold serial dilutions of Adv (10^{0.5-10.5} PFU/mL) were prepared from the concentrated and purified intact Adv. These serial diluted solutions were then subjected to long-range qPCR, binding assay, and plaque assay. It was assumed here that the levels of the intact genome, fiber, and infectivity were also divided by 10-fold in the serial dilutions. On the other hand, the DNase assay was designed to quantify the capsid-damaged Adv samples. In the case of the DNase assay, the enzyme activity of DNA degradation depending on the shielding effect of capsid proteins was measured. Thus, the intact virus samples before and after DNA extraction were exposed to the enzyme to see the change in the DNA copy number. All results were obtained from the experiments with three distinct virus samples (3 biological replications) followed by three separate qPCR measurements (3 technical replications).

2.3. The relative energy efficiency of a UV lamp

Electricity consumption for UV irradiation will vary with the application sites because of the different designs of the UV reactor. The electricity consumption obtained from laboratory experiments cannot directly be used to represent the energy consumption for field applications. A UV reactor for a laboratory experiment is designed to provide uniform UV irradiation so that the reliable values of fluorescence can be calculated (e.g., collimated beam apparatus). In contrast, a UV reactor for field application is designed to efficiently deliver the generated UV photons to the target molecules in water (e.g., cylinder shape). Although enough information for the LPUV has been accumulated from the field application to estimate the electricity consumption, the information on MPUV and microplasma UV lamp is limited because they have not been widely used in the field.

Sharpless and Linden (2005) suggested a relative energy efficiency by applying the results from a laboratory experiment with a collimated beam apparatus to estimate the electricity consumption of a UV reactor in the field. The relative energy efficiency of MPUV and microplasma UV lamps to LPUV lamps in the field scale can be estimated from the inactivation rate constant, the wall-plug efficiency, and the water factor (WF) assuming that the UV reactor designs are the same (Eq. 6).

$$\frac{E_{\text{microplasma}}}{E_{\text{LP}}} = \frac{k_{\text{LP}}}{k_{\text{microplasma}}} \times \frac{C_{\text{LP}}}{C_{\text{microplasma}}} \times \frac{WF_{\text{LP}}}{WF_{\text{microplasma}}}$$  (6)

The inactivation rate constants (cm²/mJ) were determined from the experiment for inactivation kinetics and calculated using Eqs. (7)–(9). For example, $k'$ was used for an inactivation rate constant, which was a slope of the inactivation kinetics with log $N_0/N$ as the y-axis.

$$\frac{dN}{dt} = -k'N$$  (7)

$$\ln \frac{N}{N_0} = -k't$$  (8)

$$\log_{10} \frac{N_0}{N} = k'F$$  (9)

$N$ refers to viral infectivity, and the subscript indicates exposure time. $I$ indicated the UV incidence (mW/cm²), $t$ was exposure time, and $F$ was a fluence (mJ/cm²). The wall-plug efficiencies for low and medium pressure UV were obtained from previous work Sharpless and Linden, 2005), and the one for microplasma UV was provided by the manufacturer. WF was the water factor weighed by photon energy over the germicidal wavelength (200–300 nm). According to Eqs. (10)–(11), WF is determined by a reactor design (penetration depth, $l$), water quality (UV absorbance, $a_\lambda$), lamp properties (spectral irradiance, $\rho_\lambda$). We measured the spectral irradiance for the three lamps. The penetration depth was assumed to be 5 cm for the three UV lamps based on the typical reactor design for drinking water and wastewater disinfection (Sozzi and Taghipour, 2006). Also, the UV absorbance of surface water (Thomas et al., 1996; Fig. S4) was assumed for the WF calculation. The relative energy efficiency with the inactivation rate constant, wall-plug efficiency, and WF is explained further in Section 3.4.

$$WF = \sum \rho_\lambda \times U_\lambda \times WF_k$$  (10)

$$WF_k = \frac{(1 - 10^{-\alpha})}{\alpha}$$  (11)

2.4. Statistical analysis

All statistical analysis was performed in RStudio with the data above the detection limits. Analysis of covariance (ANCOVA) was applied to compare the impact of UV irradiation to the genome, capsid protein, fiber protein, and infectivity over fluence and the impact of microplasma UV and MPUV_{223nm} over fluence. Analysis of variance (ANOVA) was conducted to compare two groups of means. The significance level was assumed to be $\alpha = 0.05$.

3. Results and discussion

3.1. Reliability of molecular assays

The long-range qPCR assay was designed to determine Adv genome integrity. The copy number from the long-range qPCR was presented with the infectivity of the initial virus solution (Fig. 2). The calibration curve showed a strong linear correlation between the initial viral infectivity and the outcome from long-range qPCR ($R^2 = 0.97$). The slope of this correlation was 1.00 in the range from 10^{0.5} to 10^{6.5} PFU/mL, suggesting the long-range qPCR has properly quantified the intact genome. Thus, the long-range qPCR was reliable in the range from about 10^{0.5} to 10^{6.5} PFU/mL, and all subsequent experiments were conducted within this range.

![Fig 2. Long-range qPCR calibration curve. The reliable range of the assay ranged from 10^{0.5} to 10^{6.5} PFU/mL.](image-url)
The DNase assay was developed to determine the viral capsid integrity. The mean diameter of DNase was estimated to be 4.2 nm (Erickson, 2009; Islan et al., 2017) based on its shape and molecular weight (Chen and Liao, 2006; Zijlstra et al., 2009). We assumed that the DNase could degrade the dsDNA of the AdV only when the virus particles have capsid damages that are greater than 4.2 nm so that the DNase can penetrate the viral capsid and degrade the viral DNA. Otherwise, the viral capsid will protect DNA from contact with DNase. For verification, two types of samples were prepared: intact AdV solution and naked DNA extracted from the AdV solution. The only difference between the two samples was the existence of the viral capsid protecting the same DNA copy number (Fig. 3). Then the two samples were exposed to either the resin alone or DNase followed by resin (which was originally supposed to quench DNase activity) to determine the function of DNase and resin for DNA degradation. In other words, DNase activity was calculated by subtracting the resin effect (R in Fig. 3) from the effect of both DNase and resin (D+R in Fig. 3) to DNA degradation. As a result, DNase activity for DNA degradation was negligible when they are protected by capsid (i.e., AdV solution). In contrast, the activity became significant (p < 0.05) when DNA was exposed to DNase (i.e., naked DNA). Therefore, DNase assay can selectively quantify the virus with the damaged capsid, which allows DNase to encounter DNA. The reliable range of DNase was assumed to be similar to that of long-range qPCR (10^{5.5}–10^{6.5} PFU/mL) because the reduction in DNA copy number was measured by long-range qPCR assay.

A binding assay was applied to determine fiber integrity, assuming AdVs with intact fibers can only bind to A549 cells and avoid being detached. The copy numbers of AdVs bound to A549 cells were measured by qPCR and presented with initial viral infectivity (Fig. 4). The log copy number of AdVs bound to the host cells linearly decreased with the initial viral concentrations from 10^{6.5} to 10^{2.5} PFU/mL and reached a constant level when the initial viral concentrations were between 10^{1.5} and 10^{2.5} PFU/mL (Fig. 4a). The reason for the constant virus copy number in the low concentration might be attributed to the non-specific binding of AdVs to host cells (Page et al., 2010). The AdV copy number was also normalized to the number of A549 cells (Fig. 4b). The two graphs in Fig 4 were similar to each other (p > 0.05), indicating that the ratio of A549 to AdV did not significantly affect the binding assay results in this range of experiments. These results can be explained by the similar numbers of A549 cells within each well (about 10^5–10^9). These numbers of cells were enough to accommodate the virus particles (less than 10^9), considering that one A549 cell can hold more than 10^6 AdVs (Wu et al., 2003).

![Fig. 4. Binding assay calibration curve shown as (a) log_{10} AdV copy number/mL and (b) log_{10} AdV/A549 copy number. The reliable range of the assay was from 10^{5.5} to 10^{6.5} PFU/mL.](image)

**3.2. AdV inactivation kinetics and mechanisms by microplasma UV**

Solutions of AdV were exposed to microplasma UV irradiation, and both the inactivation efficiency and mechanism were evaluated by the set of molecular assays, as shown in Fig. 5. The experiment was repeated with a different UV irradiance (0.10 mW/cm^2) at the center of the solution with 4 cm distance from the lamp, and the results showed no significant difference (p > 0.05) (Fig. S5). This result confirmed that the microplasma UV irradiation kinetics could be normalized by the product 1 × t referred to as fluence in this study Eqs. (7)–(9). The AdV inactivation rate constant, which was shown in the slope of the infectivity loss over the fluence, was 0.142 cm^2/mJ. Among the three viral components, the capsid damage explained the 3-log_{10} infectivity loss (p > 0.05), while genome and fiber damages were significantly different from infectivity loss (p < 0.05). The molecular assays indicated that microplasma UV irradiation mostly attacked the capsid proteins to inactivate AdV.
This observation can be explained by the overlapping of the UV absorbance of capsid proteins with the microplasma UV wavelength. These results agree with a previous study using a sodium dodecyl sulfate polyacrylamide gel electrophoresis assay to report that the monochromatic UV damaged about 40% of the hexon and penton proteins at 220 nm of 22.7 mJ/cm² (Beck et al., 2018).

Genome damage increased up to about 1 log of reduction as fluence increased until about 5 mJ/cm² before reaching saturation. This saturation was also confirmed with the naked DNA sample (Fig. S6). This plateau was attributed to the reversibility of the lesion of cyclobutane pyrimidine dimers (CPDs), the most common lesion caused by UVC irradiation. The CPDs formation follows a pseudo-zero-order reaction. In contrast, the reversal reaction conforms to a first-order reaction, so the two reactions reach an equilibrium state at a certain concentration of CPDs in the AdV genome (Errol et al., 2006).

The fiber damage was not significantly different from the negative control (p>0.05) at fluences of less than 21.2 mJ/cm². This observation means monochromatic UV at 222 nm is not effective to degrade the viral fiber proteins. A similar trend was also confirmed by Beck et al. (2018), showing fiber damage was negligible at fluences less than 22.7 mJ/cm² of monochromatic UV irradiation at 220 nm generated by a deuterium UV lamp with a bandpass filter.

The results of the molecular assays also provided knowledge on the damage patterns on AdV. These damage patterns were a unique consequence of the UV irradiation because each assay directly measured the damage on the virus particles. Therefore, the damage patterns on AdVs can play a role as a fingerprint for certain disinfectants. We confirmed that the infectivity loss by MPUV222nm was not significantly different (p>0.05) from that by microplasma UV, and the primary damage occurred to the capsid (Fig. S7). Thus, the inactivation efficacies and mechanisms by microplasma UV lamp and MPUV222nm were not significantly different even though the peak wavelength, the half-peak bandwidth, and the UV source were not the same. This approach agrees with Hull and Linden (2018), who suggested that action spectra and lamp emission spectra can be used to predict inactivation efficacy for the emerging UV sources. This phenomenon was theoretically supported by the law of photochemistry (Bolton et al., 2015). A photon that is absorbed by a molecule only causes an effective photochemical reaction. The inactivation kinetics of UV irradiation will be determined by both UV wavelength and fluence. The impact of the microplasma UV lamp and MPUV222nm on pathogens can be considered the same regardless of the UV source and experiment design. Therefore, we can conclude that the inactivation rate constants by microplasma UV are similar to those by MPUV222nm.

3.3. Effective performance of microplasma UV for water disinfection

The findings on AdV inactivation and mechanisms showed that microplasma UV and MPUV222nm have the same effects on the viruses. Based on these findings, we assume that the inactivation rate constants of bacteriophage MS2, rotavirus, and Tulate viruses by microplasma UV are the same as the constants measured previously by MPUV222nm. Table 1 lists the inactivation efficacy of AdV, bacteriophage MS2, rotavirus, and Tulate viruses by microplasma UV, MPUV222nm, and LPUV. With the assumption of the same efficacy as MPUV222nm, microplasma UV was expected to provide better inactivation of the four selected viruses than LPUV. Taking AdV as an example, the inactivation rate constant of monochromatic UV at 222 nm was 4.4 times higher than that of monochromatic UV at 254 nm (0.032 to 0.142 cm²/mJ). In agreement with our study, Linden et al. (2007) and Vazquez-Bravo et al. (2018) reported that the germicidal factor of AdV2 at 220 nm was 5 times higher than that of 254 nm. The monochromatic UV at 222 nm was confirmed to be effective to the different viruses, showing 1.2, 2.3, and 2.4 times higher inactivation rate constants for Tulate virus, MS2, and rotavirus, respectively, compared to that of monochromatic 254 nm. However, note that there could exist other viruses that are more resistant to UV at 254 nm than 222 nm.

A microplasma UV lamp generated monochromatic UV at 222 nm, primarily targeting AdV capsid proteins, while LPUV produced monochromatic UV at 254 nm mostly attacking the genome. Monochromatic UV at 222 nm was a better germicidal wavelength than that of 254 nm for inactivating a wide range of pathogens for the following two reasons. First, many viruses (MS2, T1UV, Q beta, T7m, and T7) showed higher UV absorbance at 222 nm than at 254 nm (Beck et al., 2015). UV absorbance of peptide bonds, amino acids, and proteins showed increasing tendency from 240 nm to 190 nm (Beaven and Trudel, 1952; Goodfellow and Saidel, 1951; Ham and Platt, 1952; Rosenheck and Doty, 1961). These proteins are known to be the highest molecular component for microbial cells making up over 50% of the cell while DNA makes up only 3% (Neidhardt et al., 1990). The protein contents for viruses were even higher (Laine et al., 1973), for example, AdVs were known to have more than 85% of protein contents (Piha and Green, 1965). Second, protein damages are mostly irreparable (Chondrogianni et al., 2014). In the case of DNA damage, however, the DNA repair system was well established to restore various types of UV-induced DNA lesions (Errol et al., 2006). The noticeable resistance of AdVs to LPUV was also assumed to be the viral ability to exploit a DNA repair machinery of a host cell (Gerrity et al., 2008; Ko et al., 2005).

3.4. Energy efficiency comparison among the three types of UV lamps

The energy consumption was analyzed to determine whether or not a microplasma UV lamp can be applied to UV irradiation systems for water disinfection. Energy consumption is known to be the most critical parameter for the economic and environmental impact of the UV irradiation system (Jones et al., 2018). It was estimated that the relative energy efficiency of MPUV and microplasma UV to LPUV (Eq. (6)) with a scenario where a treatment credit for viruses (4 log reduction) was assigned based solely on the UV irradiation system (USEPA, 2008). The wall-plug efficiency and water factor were determined in Section 2.3. Based on the fluence requirement for viruses, the AdV was the most resistant virus to LPUV, MPUV, and microplasma UV despite different germicidal

### Table 1

<table>
<thead>
<tr>
<th>Virus</th>
<th>Monochromatic UV at 254 nm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Monochromatic UV at 222 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>0.032 ± 0.002 cm²/mJ (125 mJ/cm²)</td>
<td>0.142 ± 0.005 cm²/mJ (26.1 mJ/cm²)</td>
</tr>
<tr>
<td>MS2</td>
<td>0.070 ± 0.002 cm²/mJ (57.1 mJ/cm²)</td>
<td>0.159 ± 0.006 cm²/mJ (25.2 mJ/cm²)</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>0.009 ± 0.004 cm²/mJ (43.0 mJ/cm²)</td>
<td>0.220 ± 0.008 cm²/mJ (18.2 mJ/cm²)</td>
</tr>
<tr>
<td>Tulate virus</td>
<td>0.158 ± 0.005 cm²/mJ (25.3 mJ/cm²)</td>
<td>0.196 ± 0.009 cm²/mJ (20.4 mJ/cm²)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Monochromatic UV at 254 nm was generated by a medium-pressure UV lamp with a bandpass filter at 254 nm.

<sup>b</sup>Experimental results from this study. Monochromatic UV at 222 nm was produced by a microplasma UV lamp.

<sup>c</sup>Monochromatic UV at 223 nm was irradiated by a medium-pressure UV lamp with a bandpass filter at 223 nm.
wavelengths of the lamps. Thus, the fluorescence requirements of MPUV and microplasma UV for viruses can be determined based on the AdV inactivation experiments similar to the fact that the fluorescence requirement of LPUV for the viruses was also decided based on the AdV inactivation experiment (USEPA, 2006). In general, LPUV was the most economical option to inactivate the three types of pathogens among the lamps (Table 2) because the wall plug efficiency and water factor of LPUV were the most favorable.

A microplasma UV lamp could be a promising option as a stand-alone water disinfection technology, where the treatment credit for viruses is assigned to UV irradiation. While the UV LED is currently considered to be the available UV lamp for the stand-alone water disinfection (Oguma et al., 2016, 2013), a microplasma UV lamp is more advantageous in terms of the relative energy efficiency. Beck et al. (2017) estimated the relative energy efficiency of UV LED to LPUV for AdV inactivation would be 90.8, 79.5, and 87.3 when 260 nm, 280 nm, and 260/280 nm wavelengths were applied, respectively. On the other hand, the relative energy efficiency of the microplasma UV lamp for AdV inactivation would decrease from 6.33 (current study with an assumption of 5 cm water depth) to 4.78, if the same water depth as that used by Beck et al. (2017) (0.6 cm) were applied. The higher energy efficiency of microplasma was previously confirmed by MS2 inactivation experiment using a KrCl excimer lamp producing 222 nm (Hull and Linden, 2018).

The improvement of the relative energy efficiency of the microplasma UV lamp is due to the wall-plug efficiency and efficient germicidal wavelength. The wall-plug efficiency of the UV LED is known to decrease as the wavelength is shortened dramatically (Chen et al., 2017). The most widely used wavelengths by UV LED lamps for water disinfection are 260 nm and 280 nm, and they had wall-plug efficiencies of 0.004 and 0.005 (Beck et al., 2017). Although a UV LED which generates far UVC (207–222 nm) was invented, its efficiencies were quite low (e.g., 3 \( \times 10^{-5} \) for 222 nm (Hirayama et al., 2009) and 10–8 for 210 nm (Taniyasu et al., 2006). On the other hand, the microplasma UV lamp already achieved a much higher wall-plug efficiency (0.015) of monochromatic wavelength at 222 nm than UV LED. In terms of the wall-plug efficiency and effective germicidal wavelength, a microplasma UV lamp seems the most advantageous option among the alternative lamps. For instance, if the wall-plug efficiency of the microplasma UV lamp increases up to 0.084, then the energy efficiency of the microplasma UV lamp becomes higher than that of LPUV. Also, the small and flat shape may have advantages when being applied for portable water purifiers.

4. Conclusions

We evaluated the microplasma UV for AdV inactivation kinetics and the lamp energy efficiency. The set of molecular assays (long-range qPCR, DNase, binding, and plaque assay) was developed to study the kinetics and mechanisms of AdV inactivation. This study presented the first dose response with viral component integrities for a viral pathogen (i.e., AdV) using the microplasma UV lamp. Microplasma UV and MPUV_{223nm} showed an insignificant difference in AdV inactivation, suggesting that the peak wavelength of monochromatic UV and the fluence are the most important parameters in designing a water disinfection system using a microplasma UV lamp. Microplasma UV (monochromatic UV at 222 nm) showed 4.4 times higher inactivation rate constant than that of LPUV. The microplasma UV was expected to be effective for other types of viruses due to its ability to target the capsid proteins instead of the genomes. Although the current wall-plug efficiency would delay applying the microplasma UV lamp for water disinfection, the efficient wavelength could facilitate the application of microplasma UV lamps as the technology improves.

We identified the following research that could further contribute to the potential application of microplasma UV in water and wastewater disinfection. First, energy efficiency could be improved by enhancing the wall-plug efficiency of the microplasma UV lamp and finding the optimal design shape of the microplasma UV lamp (e.g., small and thin shape) (Chen et al., 2017). Second, a life cycle assessment for the UV irradiation system can be conducted for better evaluation. Even though energy consumption was assumed to be the most critical parameter for the UV irradiation system, the other consumables would significantly impact the environmental footprint. For example, microplasma UV lamps do not require toxic chemicals such as Hg for the filling gas. On the other hand, the lamp price is currently relatively high compared to the other types of UV lamps. Thus, the lamp price should be considered in designing a water treatment system. Third, noticeable improvement in virus inactivation might allow the UV irradiation to be free from other disinfectants as secondary disinfectants. For instance, the chlorination is currently required not only for residual disinfectant but also for virus inactivation. As a stand-alone technology, microplasma UV irradiation can be compared to the LPUV supported by chlorination. Finally, since UV at 222 nm is near the vacuum UV region (< 200 nm), where various radicals could be produced depending on the water matrix, further study is necessary to evaluate the potential formation of toxic disinfection byproducts by microplasma UV irradiation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project was supported by grant R835826 from the U.S. Environmental Protection Agency (EPA). Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the EPA. Further, the EPA does not endorse the purchase of any commercial products or services mentioned in the publication. The microplasma lamp and its functional components were generously provided Dr. Cy Herring from EP Illumination and Professor J. Gary Eden from the University of Illinois at Urbana-Champaign.

Supplementary materials


References
