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Inactivation of Viruses on Surfaces by Ultraviolet Germicidal Irradiation

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In many outbreaks caused by viruses, the transmission of the agents can occur through contaminated environmental surfaces. Because of the increasing incidence of viral infections, there is a need to evaluate novel engineering control methods for inactivation of viruses on surfaces. Ultraviolet germicidal irradiation (UVGI) is considered a promising method to inactivate viruses. This study evaluated UVGI effectiveness for viruses on the surface of gelatin-based medium in a UV exposure chamber. The effects of UV dose, viral nucleic acid type (single-stranded RNA, ssRNA; single-stranded DNA, ssDNA; double-stranded RNA, dsRNA; and double-stranded DNA, dsDNA), and relative humidity on the virus survival fraction were investigated. For 90% viral reduction, the UV dose was 1.32 to 3.20 mJ/cm² for ssRNA, 2.50 to 4.47 mJ/cm² for ssDNA, 3.80 to 5.36 mJ/cm² for dsRNA, and 7.70 to 8.13 mJ/cm² for dsDNA. For all four tested viruses, the UV dose for 99% viral reduction was 2 times higher than those for 90% viral reduction. Viruses on a surface with single-stranded nucleic acid (ssRNA and ssDNA) were more susceptible to UV inactivation than viruses with double-stranded nucleic acid (dsRNA and dsDNA). For the same viral reduction, the UV dose at 85% relative humidity (RH) was higher than that at 55% RH. In summary, results showed that UVGI was an effective method for inactivation of viruses on surfaces.

Keywords bacteriophage, inactivation, surfaces, UVGI, virus

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INTRODUCTION

Viruses are obligate parasites that cannot multiply or propagate outside specific host cells. In the environment, surfaces become contaminated with viruses through contact with infectious body fluids or the settling of airborne viral particles. For surfaces to serve as sources of viral disease, the involved virus must be able to survive in association with the surface until it encounters a susceptible host. There have been a number of viral outbreaks related to surface-related transmission, such as hepatitis virus,^(1,2) rotavirus,^(3,4) enterovirus,^(5,6)

and severe acute respiratory syndrome coronavirus (SARS CoV).^(7,8)

Recently, enteric viruses and SARS CoV have emerged as major public health issues due to their ability to spread through close person-to-person contact and their transmission by droplets generated by an infected person. Enterovirus and SARS CoV could also be spread when a person touches a surface contaminated with infected droplets and then touches their nose, mouth, or eyes. In Taiwan, enterovirus 71 caused 78 deaths in a large outbreak in 1998, and reoccurred in recent years.^(5,6) The outbreak was highly related to contact transmission and resulted in the widespread of hand-foot-and-mouth disease among exposed population. For SARS CoV, the outbreak occurred in Taiwan with 73 deaths in 2003. Some evidence revealed that polymerase chain reaction (PCR) positive swab samples were recovered from frequently touched surfaces in rooms occupied by SARS patients and in nurse stations used by staff.^(9,10) These observations led to speculation that a possible route of SARS CoV transmission was contact with environmental surfaces.

There are many control techniques that could reduce risk from viral infection on surfaces, including heating sterilization,⁽¹¹⁾ ultraviolet germicidal irradiation (UVGI),⁽¹²⁾ and chemical disinfectants.⁽¹³⁾ However, many surface materials cannot be heat sterilized and might be damaged by chemical disinfection.⁽¹⁴⁾

In contrast with most disinfectants, UVGI has been well recognized as an effective method for inactivating microorganisms.^(12,15–18) The mechanisms of UVGI on microorganisms are uniquely vulnerable to light at wavelengths at or near 253.7 nm because the maximum absorption wavelength of a DNA molecule is 260 nm.⁽¹⁹⁾ After UV irradiation, the DNA sequence of microorganisms can form pyrimidine dimers, which can interfere with DNA duplication, as well as lead to destruction of nucleic acids and render the viruses noninfectious.⁽²⁰⁾

In addition, UVGI effectiveness for microorganisms inactivation was related to irradiation level, duration of irradiation, and relative humidity (RH).^(21–23) Until now, the UVGI virus-related investigations evaluated only viruses in water^(24–26) and air;⁽²⁷⁾ these studies found that UVGI would effectively inac-

tivate viruses in water and in their airborne phases. Moreover, the type of viral nucleic acid, host cell repair mechanisms, and capsid structure of virus played an important role in virus inactivation.⁽²⁶⁾ However, there is little information available on the effectiveness of UVGI for inactivation of viruses on surfaces.

The purpose of this study was to determine the effectiveness of UVGI for virus inactivation on surfaces. For selection of virus target, it is believed that radiation would restructure the nucleic acid of the microorganisms and destroy its replication ability; therefore, the type of the viral nucleic acid may play a critical role on virus inactivation by UVGI.

According to the types of the nucleic acids, viruses can be divided into four groups, including single-stranded RNA (ssRNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), and double-stranded DNA (dsDNA). In addition, bacteriophages are more resistant to UVGI than other pathogenic viruses; therefore, they are considered as suitable indicators. The bacteriophages used in this study have been used as indicators of poliovirus, enterovirus, enveloped viruses, and human immunodeficiency virus.^(28–30) Consequently, this study evaluated the effects of UV dose, different nucleic acid type of virus (four different bacteriophages with ssDNA, ssRNA, dsDNA, and dsNA), and RH (55% and 85%) on virus survival fraction after UVGI exposure.

METHODS

Test Viruses

In medical and environmental virology applications, bacteriophages have widely served as suitable surrogates for mammalian viruses.^(28–30) In this study, the tested viruses were four different bacteriophages: ssRNA (MS2, American Type Culture Collection, ATCC 15597-B1), ssDNA (phi X174, ATCC 13706-B1), dsRNA (phi 6 with envelope lipid, ATCC 21781-B1), and dsDNA (T7, ATCC 11303-B1). The host bacteria were *Escherichia coli* F-amp (ATCC 15597) for MS2, *E. coli* CN-13 (ATCC 13706) for phi X174, *E. coli* 11303 (ATCC 11303) for T7, as well as *Pseudomonas syringae* (ATCC 21781) for phi 6.

In the current study, a high titer stock of bacteriophages (10^9 – 10^{10} PFU/ml, where PFU is plaque forming units) was prepared via plate lysis and elution.⁽³¹⁾ Moreover, the plaque assay⁽³²⁾ for determining virus infectivity and phage cultivation methods were all followed from the ATCC product information sheet. To allow the phage to attach to the host, the bacteriophages were mixed with their own respective host.

First, 5 mL molten top agarose (containing only 0.7% agarose) was added to a sterile tube of infected bacteria. The medium for MS2, phi X174, T7, and phi 6 phage cultivation included Luria-Bertani agar (244520; Difco Laboratories, Detroit, Mich.), nutrient agar (213000; Difco) with 0.5 NaCl, trypticase soy agar (236950; Difco), and NBY agar (containing nutrient broth, yeast extract, K_2HPO_4 , KH_2PO_4 , and

$MgSO_4 \cdot 7H_2O$), respectively. Then the contents of the tube were mixed by gentle tapping for 5 sec and poured onto the center of a labeled agar plate.

Finally, the plate was incubated for 24 hr either at 37°C for coliphages or at 26°C for phi 6. After cultivation, 5 mL SM buffer (containing NaCl, $MgSO_4 \cdot 7H_2O$, Tris, and gelatin) was pipetted onto a plate that showed confluent lysis. Then the plate was slowly rocked by a mechanical shaker (model OS701, TKS Orbital Shaker; Taipei, Taiwan), for 40 min and the buffer was transferred to a tube for centrifugation in a Kubota centrifuge (Kubota Corporation, Tokyo, Japan) at $4000 \times g$ for 10 min. After the supernatant was removed, the remaining phage stock was kept at $-80^\circ C$. From our preliminary results (data not shown), virus infectivity could be maintained for 24 hr at 4°C. For UVGI experiments, the virus titers were determined by plaque assay, and the virus suspension was stored at 4°C within 24 hr.

Surface Test System

Gelatin-Based Medium

From an earlier study,⁽¹²⁾ there is little data available for virus inactivation by UVGI on surfaces. Another study⁽³³⁾ showed that different kinds of surface compositions to which viruses were adsorbed may cause viruses to lose their infectivity because of desiccation. Therefore, the stability of virus infectivity on the evaluated surface is very important. In the current study, a gelatin-based medium was used for the tested surface because it offered a more ideal growth medium for the viruses, preserving their infectivity. From our preliminary tests (data not shown), virus infectivity remained the same at least for 1 hr at RH 55% and 85% (with coefficient of concentration variation below 20%).

The gelatin-based medium was composed of LB (Luria-Bertani) broth with 7% gelatin. After sterilization by autoclave (121°C, 15 min) the medium solidified at 4°C. Then, a diluted culture of virus stock solution (10^9 PFU/mL, 0.1 mL) was spread on the surface of the gelatin medium and naturally air dried (20 min) in a laminar flow hood to prevent contamination. On each plate, the virus concentration was 10^8 PFU/mL. After UVGI exposure, the gelatin-based medium was directly liquefied at 37°C in a incubator (model LT1601; TKS Technology, Taiwan) for further quantification without elution procedure. Finally, the viral particles in the liquid phase of the medium were subjected to plaque assay for coliphages at 37°C and for phi 6 at 26°C. Experiments were performed at least in triplicate for each set of conditions with different UV dose.

All plates (both UV-exposed and UV-unexposed) were incubated for 24 hr. The virus survival fraction was calculated as the ratio of the number of plaques forming on the UVGI-exposed plates compared with the number of plaques on the UVGI-unexposed control plates. All experiments were conducted in darkness to prevent visible light effects.⁽³⁴⁾ The test system was located in a chemical hood so that the exhausted gas was vented outside.

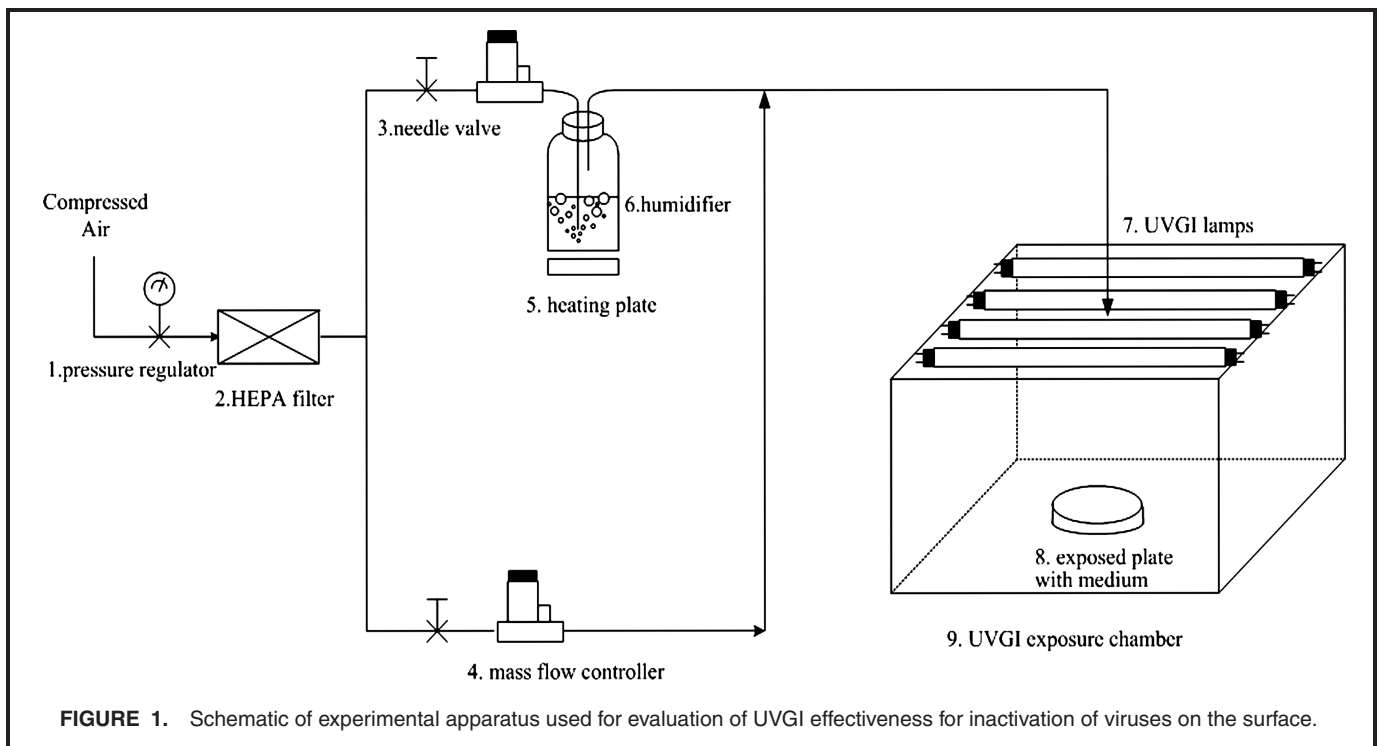


FIGURE 1. Schematic of experimental apparatus used for evaluation of UVGI effectiveness for inactivation of viruses on the surface.

RH Regulation Unit

A humidified gas stream was generated by passing pure compressed air through a humidity saturator (Figure 1). Sterile deionized water was used in the saturator. Water vapor content (i.e., RH) in the gas stream was adjusted by changing the flow rate ratio of the humidified gas stream to a dry gas stream and was finally measured using a hygrometer (Sekunden-Hygrometer 601; Testo, Lenzkirch, Germany) placed in the UV exposure chamber. The gas flow rates in the chamber was 10 L/min. For evaluating the effect of RH, the humidified gas stream was heated by adding a dry gas stream to reach a medium (RH 55%) or very humid condition (85%) at 25–28°C.

UV Exposure Unit

The UV exposure chamber was approximately 0.02 m³ in volume (0.27 m × 0.30 m × 0.3 m). The exposed samples were irradiated with four 8W UV-C lamps (germicidal lamp, TUV 8W/G8 T5; Philips Electronic Instruments, Eindhoven, The Netherlands) with a radiation peak at 253.7 nm for germicidal action. The lamps were placed 30.5 cm above the surface of the medium and were wrapped in a layer of cellophane to attenuate original irradiation magnitude. The intensity of UVGI on the surface of the medium was measured using an UV-radiometer (P-97503-00; Cole-Parmer, Vernon Hills, USA) with a 254-nm sensor.

In a preliminary study (data not shown), UV intensity in the range of 60 to 240 μW/cm² was used, and the exposure time varied from 3 sec to 6 min. Because the UV dose is the product

of the UV intensity and UV exposure time, the evaluated UV doses were in the range of 0.18 to 86.4 mJ/cm². In the current study, experiments were performed at least in triplicate for each set of conditions for UV intensity (120 μW/cm²), exposure time (5, 15, 35, 85, 165, 255 sec), RH (55% and 85%), and tested virus (MS2, phi X174, T7, and phi 6).

Survival Fraction of Viruses vs. UVGI Exposure

The total dose to which a virus on a surface was exposed was defined as the product of the UVGI intensity I on the viruses and the exposure time t . The survival fraction is the ratio that represents the virus concentration after UVGI exposure. Microorganisms susceptibility factor (K -value) was derived from the exponential decay model presented in following equation:

$$\frac{N_{uv}}{N_0} = e^{-KI t} \quad (1)$$

where

N_{uv} = concentration of virus surviving after exposure to UVGI (PFU/mL)

N_0 = concentration of virus unexposed to UVGI (PFU/mL)

I = UV intensity (μW/cm²)

t = UV exposure time (sec)

K = microorganism susceptibility factor (cm²/mJ)

Statistical Analysis

The log survival data vs. UV dose for each experiment was used to perform regression analysis on the data for each virus. R² values were obtained by regression analysis. Generation of

regression lines and prediction of the doses required for 90% and 99% viral reduction were accomplished by including data points from all experiments for each tested virus. Comparisons of survival fraction among the viruses were performed using *t* test to evaluate statistically significant differences ($p < 0.05$).

RESULTS

Results of the survival fraction of four bacteriophages at two RH conditions are presented in Figures 2 and 3. The survival fractions of all four viruses were found to be inversely related to UV dose. For 90% viral reduction, the ssRNA virus (MS2) required an extremely low UV dose (1.32 to 3.20 mJ/cm²), ssDNA virus (phi X174) was more resistant than MS2 and required a higher UV dose (2.50 to 4.47 mJ/cm²), dsRNA (phi 6) required a relatively higher dose (3.80 to 5.36 mJ/cm²), and dsDNA virus (T7) required a high dose (7.70 to 8.13 mJ/cm²).

These results clearly indicate that dsRNA and dsDNA viruses are more resistant to UVGI than those of ssRNA and ssDNA viruses (UV doses for dsRNA and dsDNA was approximately 3 times higher than those for ssRNA and ssDNA, $p < 0.05$). For 99% viral reduction, the UV dose for MS2 ranged from 2.51 to 6.50 mJ/cm², for phi X174 from 5.04 to 8.34 mJ/cm², for phi 6 from 7.75 to 10.57 mJ/cm², and for T7 from 15.54 to 16.20 mJ/cm². These results indicate that the dose for viral reduction of dsRNA and dsDNA viruses on a surface is approximately 3 times higher than those of ssRNA and ssDNA viruses ($p < 0.05$).

Based on exponential decay model, the microorganism susceptibility factors, *K* value, varied widely. *K* values of ssRNA/DNA viruses were higher than those of dsRNA/DNA viruses. This could be because dsRNA/DNA viruses are more resistant to UV irradiation than ssRNA/DNA viruses. For the

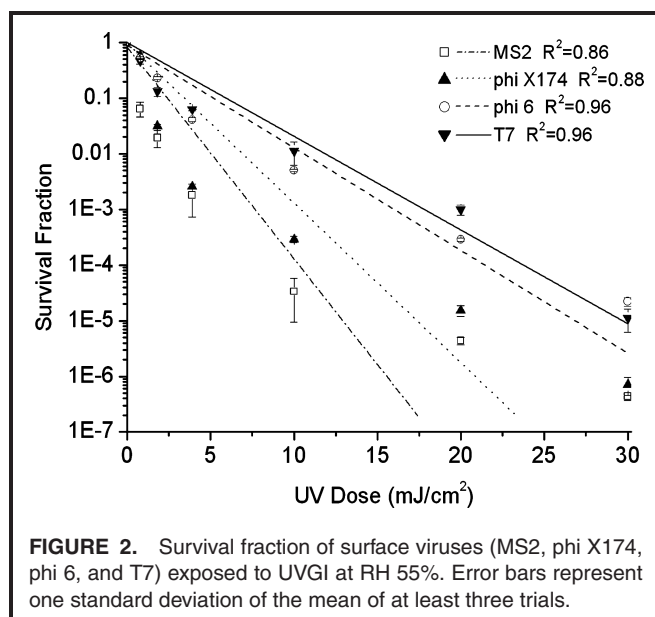


FIGURE 2. Survival fraction of surface viruses (MS2, phi X174, phi 6, and T7) exposed to UVGI at RH 55%. Error bars represent one standard deviation of the mean of at least three trials.

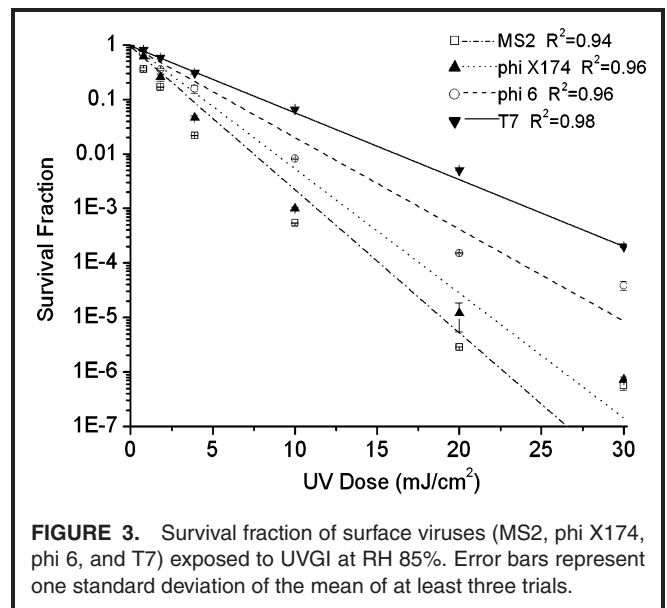


FIGURE 3. Survival fraction of surface viruses (MS2, phi X174, phi 6, and T7) exposed to UVGI at RH 85%. Error bars represent one standard deviation of the mean of at least three trials.

four types of viruses, *K* values (0.27–0.64 cm²/mJ) at 85% RH were lower than those (0.3–0.83 cm²/mJ) at 55% RH (Figures 2 and 3), which demonstrates that a higher UV dose is required to inactivate viruses at higher RH ($p < 0.05$). This finding was in agreement with our previous bacterial and fungal findings.⁽²²⁾

These results could be explained by possible water sorption onto viruses, which provides protection against UV-induced DNA or RNA damage at higher RH. Moreover, the RH effects on UVGI effectiveness were also related to the type of virus nucleic acid. The RH effects on UVGI inactivation of ssRNA and ssDNA viruses on surfaces were greater than those of dsDNA and dsRNA.

The effectiveness of UVGI for viruses inactivation on surfaces was fitted well with an exponential decay model. Moreover, observations are in agreement with the Bunsen-Roscoe reciprocity law⁽³⁵⁾ that states if a photobiologic effect depends purely on photochemical events, the biologic effect of a UV exposure depends on the product of the irradiance and exposure time. In summary, the UVGI effects for virus inactivation on surface depended on UV dose and percent RH. For all nucleic acid types of virus, the survival fraction decreased exponentially at higher UV dose.

DISCUSSION

In air, an airborne virus with dsDNA (adenovirus) was less susceptible to UVGI than viruses with ssRNA (Coxsackie B1 virus, Influenza A virus, Sindbis virus and Vaccinia virus).⁽²⁷⁾ In suspension, MS2 has higher resistance to UVGI than other ssRNA viruses (feline calicivirus, Ecovirus, Coxsackie virus, and poliovirus) or dsDNA virus (PRD1).^(24–26) MS2 was more susceptible to UVGI than those of phi 6 and T7 because the complex nucleic acids (doubled strained genomes) of both phi 6 and T7 could enable these two phages to use the host enzymes to repair damages. In addition, bacteriophages are more resistant to UVGI than other pathogenic viruses in the

environment. Therefore, these viruses may be less susceptible to UVGI inactivation than the bacteriophages used in this study.

In previous investigations,^(16–17) solid media that included beef extract agar, beef-infusion blood agar, and malt extract agar were widely used for inactivation of microorganisms on surfaces by UVGI. Regarding inactivation of other microorganisms on surfaces, the UV doses for 90% viral reduction were similar to those for *E. coli*, *Serratia marcescens*, *Staphylococcus haemolyticus*, *Salmonella typhi*, *Streptococcus viridans*, *Staphylococcus albus*, *Shigella paradysenteriae*, and yeast (1.7–7.4 mJ/cm²) but much lower than for *Bacillus subtilis* (19 mJ/cm²) and *Penicillium citrinum* (22 mJ/cm²).

These findings revealed that virus susceptibility to UVGI was similar to that of nonsporulating species, such as fragile bacteria and yeasts, but is higher than that for endospore-forming bacteria and fungal spores. Moreover, the susceptibility of microorganisms to UV irradiation was highly related to the presence or absence of a cell wall, cell wall thickness, and the type of nucleic acid.

In comparison with airborne evaluation,⁽³⁶⁾ it was demonstrated that UV lethal radiation doses required for airborne viruses were lower than those for viruses on surfaces. Furthermore, the ratio of the 90% viral reduction dose for virus on surfaces to airborne viruses ranged from 3.9 to 7.6 for MS2, from 5.6 to 9.0 for phi X174, from 5.7 to 6.2 for phi 6, and from 6.8 to 8.5 for T7. This may be explained by the fact that viruses can form aggregation on surfaces. When compared with studies where UVGI effectiveness was investigated on viruses in suspension, much higher UV doses were needed for 90% inactivation of MS2 virus (12–24 mJ/cm²) than on a surface (1.32 to 3.2 mJ/cm²).^(25,26) Viruses may be less susceptible to UVGI when associated with water.

This study used gelatin-based medium as the test surface for evaluating the susceptibility of viruses to UVGI. Gelatin is a protein source and solidifying agent for use in preparing microbiological culture medium. The smooth surface of this medium is suitable for viruses to preserve their infectivity. In UVGI applications, the UVGI susceptibility of viruses may be changed because of different kinds of surface compositions to which viruses are adsorbed. Viruses may be more susceptible to UVGI on the growth media because of desiccation.

When considering UVGI application to inactivate viruses on the surface, care needs to be taken, since it is known that microorganisms' growth could occur in crevices, and UVGI cannot completely penetrate these shadowed areas. Moreover, UVGI could damage or discolor surfaces and cause possible health effects, such as erythema of the skin and photokeratitis; therefore, humans, plants, and animals should be removed from the area when UVGI is applied. In summary, the effectiveness of UVGI for viral reduction on surfaces may be associated with the type of virus nucleic acid. Viruses with dsRNA or dsDNA may be less susceptible to UVGI inactivation. At high RH, a higher UV dose was required to inactivate virus on surfaces.

CONCLUSIONS

The effects of UV dose, type of virus nucleic acid, and RH on the effectiveness of UVGI to inactivate surface viruses were evaluated in a UV exposure chamber. For virus inactivation on the surface, the effectiveness of UVGI strongly depended on a type of virus nucleic acid. Viruses with dsRNA or dsDNA could be less susceptible to UVGI inactivation. For 90% surface virus inactivation, the UV dose for dsRNA and dsDNA viruses was approximately 2 to 3 times higher than ssRNA and ssDNA viruses, respectively. The susceptibility factor for the viruses was higher at 55% RH than at 85% RH possibly because when RH increases, water sorption onto the virus surface might provide protection against UV-induced DNA or RNA damage.

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