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Development and verification of a test rig for inactivation of bacteria and (corona-) viruses by UVC air disinfection systems

Abstract: The ongoing coronavirus pandemic spreads through airborne transmission and is therefore difficult to contain. However, coronaviruses are highly sensitive to UVC, so UVC air disinfection systems should be able to inactivate the virus. Unfortunately, so far there are only few possibilities to test the reduction of airborne viruses or other pathogens. A special test rig, which mainly consisted of a nebulizer and an airflow system, was developed to determine the antiviral and antibacterial efficiency of UVC air disinfection systems. In the assessment of such an UVC air disinfection system with nebulized *Staphylococcus carnosus* and a sampling period of 30 minutes, a mean bactericidal reduction of 3.70 log₁₀ (99.98 %) was determined. For antiviral irradiation of the coronavirus surrogate phi6 a mean viral load reduction of 1.18 log₁₀ (93.40 %) was observed after a sampling period of 10 minutes. Therefore, mobile UVC air disinfection systems could be applied in hospitals, retirement and nursing homes.

Keywords: SARS-CoV, SARS-CoV-2, coronavirus, Covid-19, phi6, *S. aureus*, bacteria, *Staphylococcus aureus*, UVC, UV-C, ultraviolet, irradiation, inactivation, disinfection

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1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the dangerous lung infection Covid-19, continues to spread, resulting in nearly 166 million confirmed infections and more than 3.44 million deaths worldwide [1]. The main route of transmission is absorption of the airborne infectious particles through the mucous membranes or conjunctiva [2,3]. According to Hessling et al. there is an increasing evidence that coronaviruses are highly sensitive to ultraviolet radiation between 200 – 280 nm (UVC) [4]. For

inactivation of airborne coronavirus there are very few publications. Walker and Ko demonstrated the inactivation of murine hepatitis virus (MHV) coronavirus at 254 nm with an AGI-30 liquid impinger and a 90 % reduction dose (D90) of 0.56 mJ/cm² (relative humidity (RH): 50 %) [5]. Buonanno et al. observed inactivation of airborne human coronavirus (HCoV-OC43 and HCoV-229E) at a wavelength of 222 nm and a D90 of 0.39 mJ/cm² and 0.56 mJ/cm² (RH: 66 %) [6]. Qiao et al. evaluated the influence of UVC to porcine respiratory coronavirus (PRCV) in a flow tube reactor and observed a D90 of 8.53 mJ/cm² (RH: 57 – 62 %) [7]. It should be noted that over all aerosol studies, there are only limited few UVC irradiation investigations of virus and bacteria available. For instance Mc Devitt et al. and Jensen et al. determined the D90 inactivation of influenza virus to be 1.05 mJ/cm² (RH: 81 % – 84 %) and 1.93 mJ/cm² (68 % RH) [8,9]. The gram-positive staphylococci species *Staphylococcus aureus* (*S. aureus*) represent bacterial pathogens, which are notorious agents for nosocomial infections [10]. Kozajda et al. detected in several investigations the airborne pathogen *S. aureus* in different environments [11]. VanOdsell et al. and Nakamura et al. performed aerosol inactivation experiments with staphylococcus spp. and observed a D90 of 1.40 mJ/cm² and 2.00 mJ/cm² [12,13].

Besides these results, the UVC inactivation in airborne state of pathogens has not yet been studied to a sufficient extent. Regarding the airborne transmission of the coronavirus pandemic, this would be of major interest. Unfortunately, due to regulatory issues, most laboratories are not permitted to perform these investigations. However, bacteriophages are considered to be suitable surrogates for some human or animal viruses [14]. The bacteriophage phi6 has been previously discussed as an useful surrogate of comparable structure, particle size, and environmental stability to coronaviruses [14–16]. The enveloped virus exhibits a diameter of 75 nm with an RNA genome of 13.5 kbp [19]. Therefore, the investigations are carried out with the enveloped dsRNA virus phi6 [17], a member of the *Cystoviridae* [18]. For the antibacterial investigations *Staphylococcus carnosus* (*S. carnosus*), a non-pathogenic relative of the methicillin-resistant *Staphylococcus aureus* (*S. aureus*) will be used.

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2 Materials and Methods

2.1 Test rig

A special test rig, which mainly consisted of a nebulizer and an airflow system, was developed to determine the antiviral and antibacterial efficiency of a commercial UVC air disinfection system (airtubeUVC40_desk of UVCclean, Owen, Germany). The test rig is described in detail in Figure 1. The nebulized pathogens enter the mixing chamber to separate the heavier droplets. Through the volume flow generated by the fan the aerosol particles reach the UVC air disinfection system. By selective sampling before and after entering the UVC air disinfection system, the survival rate can be determined. After additional filtering, the airflow is passed to an air purifier to avoid environmental contamination. The air disinfection system has a diameter of 170 nm and a length of 460 mm and consists of a 60 W low pressure mercury lamp.

Figure 1: Developed test rig including the UVC air disinfection system for the antiviral and antibacterial investigations.

2.2 Pathogens and Medium

2.2.1 *S. carnosus*

Due to safety regulations in the available laboratory, it is not possible to cultivate pathogens like *S. aureus*. Therefore, the test organism for the antibacterial experiments was the non-pathogenic relative *S. carnosus* (DSM 20501). This strain was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) and cultivated in tryptic soy yeast extract medium (M92). The growth medium consisted of 30 g tryptic soy broth, 3 g yeast extract and 1000 g distilled water. The pH was adjusted to 7.0 – 7.2. Prior to irradiation, *S. carnosus* was cultivated in this growth medium for 5 – 6 h at 37 °C on a rotary shaker at 170 rpm. In the next step, the medium was removed and the resultant pellet was washed in phosphate buffered saline (PBS). Resuspended cells were diluted in the specific solution for the certain irradiation experiment until a starting concentration of about 1×10^9 colony forming units (CFU) per mL was reached for experimental use. Diluted bacteria suspensions were serially plated out over at least three powers of ten and at least three independent experiments were performed.

2.2.2 Bacteriophage phi6

Bacteriophage phi6 (DSM 21518) and its host *Pseudomonas syringae* (DSM 21482) were obtained from Leibniz-Institute DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). *P. syringae* was propagated in Tryptic Soy Broth (TSB, Sigma-Aldrich, St. Louis, USA). For preparation of host cells, 25 mL of TSB were inoculated with a single bacterial colony and the culture was grown overnight at 170 rpm and 25 °C in order to obtain an optical density at 600 nm of 0.20 – 0.25. This was equal to $1 - 5 \times 10^8$ colony-forming units (CFU) per mL. The preparation by plate lysis and elution of a high titer phage stock solution was performed according to [20]. After irradiation, the number of active phages was determined using the double agar overlay plaque assay [21]: 100, and 200 µL of sequentially diluted phi6 samples in saline magnesium gelatin buffer (SMG), 100 µL of host bacteria *P. syringae*, and 3 mL of soft agar were mixed and plated over TSB agar plates. Plaques were counted after incubation for 24 h at 25 °C and phage concentration was expressed in plaque forming units (PFU) per mL. At each sampling time point, three technical replicates were measured, and at least three independent experiments were performed for each condition.

3 Results and Discussion

3.1.1 Technical data of the UVC disinfection system

The measured emission peak for the 60 W mercury low pressure lamp was 253.7 nm and the mean irradiation intensity within the airflow 14.68 mW/cm². The associated flow rate was 40 m³/h and the calculated mean irradiation time was 0,3 s. Therefore, the irradiation dose of the passing air is estimated to be 5,81 mJ/cm² and applied to determine the D90 doses of *S. carnosus* and phi6 and compare them with the literature.

3.1.2 Antibacterial investigations

During the bacterial investigations, there was a mean increase in the RH from 58.67% to 99.00% (+ 40.33 %) and a decrease in temperature from 20.1 °C to 18.2 °C. The antibacterial effect on airborne *S. carnosus* is successfully demonstrated in Figure 2 A). For UVC off, the reduction was 1.40 log₁₀, 0.95 log₁₀ and 1.15 log₁₀ averaged to 1.12 log₁₀ at sampling for 30 min. For UVC on, an antibacterial effect of 3.36 log₁₀, 3.74 log₁₀ and 3.76 log₁₀ were demonstrated. The averaged decrease is 3.70 log₁₀ (99.98%) during a sampling of 30 minutes. The subsequently calculated survival rate of *S. carnosus* after subtracting the unirradiated reference is 2.59 log₁₀ (99.74 %). With the assumed irradiation dose of 5.81 mJ/cm² the *S. carnosus* log reduction dose would be 2.24 mJ/cm², which fits well to be published D90 values of 2.00 mJ/cm² for inactivation of *S. aureus* and 1.40 mJ/cm² for *S. epidermidis* [12,13].

3.1.3 Antiviral investigations

The average RH for the viral experiments was between 56 % and 73 % (+17 %), while the temperature decreases of 1.37 °C. The inactivation of the airborne coronavirus surrogate phi6 was demonstrated and is presented in Figure 2 B). After reference sampling of 10 minutes with UVC off, a reduction of 0.33 log₁₀, 0.49 log₁₀, 0.22 log₁₀ and averaged 0.35 log₁₀ was observed. Within the irradiation experiments (UVC on) the mean observed virus load reduction was 1.23 log₁₀, 1.05 log₁₀, 1.23 log₁₀ and collectively 1.18 log₁₀ (93.40 %). After subtraction, the unirradiated reference, the comparative evaluation result was 0.83 log₁₀ (85.10 %). With the assumed irradiation of 5.81 mJ/cm² the subsequently determined D90 was 7,00 mJ/cm². Besides the airborne investigation by

Tseng et al. for dsRNA phi6 and in aerosol state data are very limited. The associated D90 for phi6 is 0.54 (RH: 55 %) and 0.74 mJ/cm² (RH: 85 %) [22]. Walker and Ko observed for MS2 inactivation a D90 of 2.61 mJ/cm² (RH: 50 %) [5], contrary to Tseng et al. who determined a D90 of 0.28 mJ/cm² (RH: 55%) and 0.36 mJ/cm² (RH: 85%) for MS2, respectively [22]. Therefore, the D90 for virus inactivation of Tseng et al. are probably not comparable to the results obtained here. A potential reason could be the use of different technical setups and microbiological methods. Tseng et al. applied several UVC lamps, as well as the impaction collection method (Andersen sampler). Buonanno et al. and Walker et al. observed lower D90 for the inactivation of MHV and HCoV-OC43 and HCoV-229E, compared to the coronavirus surrogate phi6. It should be noted that Buonanno et al. used 222 nm radiation for their investigations. For the inactivation of the PRCV a mean D90 of 8.53 mJ/cm² (RH: 57 – 62 %) was determined [7], which is slightly above the calculated D90 value of 6,83 mJ/cm² in this research. Two of the most important factors for the deviations could be the use of the porcine coronavirus strain and the application of different technical setups.

Figure 2: Comparison of the results of the antibacterial and antiviral aerosol investigations, measured before and after entering the UVC air disinfection system. In A) the *S. carnosus* results in CFU/mL for the reference experiments without UVC irradiation, UVC irradiation and the subtracted difference between reference (UVC off) and irradiation (UVC on). B) phi6 results in PFU/mL with reference, irradiation, and the subtracted difference.

4 Conclusion

The developed test rig is capable to prove and even quantify the reduction of airborne pathogens by UVC air disinfection systems including coronavirus surrogates and probably even coronaviruses. The coronavirus surrogate phi6 seems to be at least equally or probably more resistant to UVC irradiation than coronaviruses. From our perspective, UVC air disinfection systems could bring new bright light to contain the coronavirus pandemic to public healthcare institutions. The next step should be similar UVC investigations on coronaviruses, which cannot be performed in our laboratory.

Author Statement

Conflict of interest: The author state no conflict of interest.
Ethical approval: The conducted research is not related to either human or animals use.

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