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WO_3 photocatalyst containing copper inactivates SARS-CoV-2 Pango lineage A and Omicron BA.2 variant in visible light and in darkness

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019, which has been a global pandemic. Since SARS-CoV-2 is transmitted through contaminated surfaces and aerosols, environmental disinfection is important to block the spread of virus. Photocatalysts are attractive tools for virus inactivation and are widely used as air purifiers and coating materials. However, photocatalysts are inactive in the dark and some of them need to be excited with a light of specific wavelength. Therefore, photocatalysts that can effectively inactivate SARS-CoV-2 in indoor environments are needed. Here we show that a WO_3 photocatalyst containing copper inactivated the SARS-CoV-2 WK-521 strain (Pango lineage A) upon irradiation with white light, in a time- and concentration-dependent manner. Additionally, this photocatalyst also inactivated SARS-CoV-2 in dark condition, due to the antiviral effect of copper. Furthermore, this photocatalyst inactivated not only the WK-521 strain but also the Omicron variant BA. 2. These results indicate that the WO_3 photocatalyst containing copper can inactivate indoor SARS-CoV-2 regardless of the variant, in visible light or darkness, making it an effective tool for controlling the spread of SARS-CoV-2.

Keywords: SARS-CoV-2 inactivation; Pango lineage A; Omicron variant BA. 2; WO_3 photocatalyst; time-dependency; dose-dependency; copper based disinfection; environmental disinfection

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19), which has had an unprecedented impact on modern human civilization [1], and resulted in more than 6.3 million deaths globally, as of early June 2022. Despite the development of drugs and vaccines, the number of infected people continues to increase. Although the transmission route of SARS-CoV-2 is still being debated, it is generally believed to be transmitted through the airborne route, surface contamination, and fecal-oral transmission [2]. Thus, inactivation of the virus in the air and on surfaces is essential for controlling its transmission. In addition, the genome of SARS-CoV-2 has mutated rapidly, and several variants are reported. Mutations in virus help it to evade the host immune system and to acquire drug resistance. Therefore, despite the presence of vaccines and drugs, it is important to find effective ways to inactivate the virus to prevent the spread of infection, regardless of the variant. It is reported that SARS-CoV-2 can be inactivated by photocatalysts [3], heat [4], ultraviolet (UV) [5,6] and disinfectants such as ethanol [7]. Especially, since photocatalysts are harmless to human body unlike UV, they have recently received great attention and it is proposed that they can be applied for disinfection of living and working spaces, without evacuating people.

Photocatalysts are excited by light and exhibit a strong oxidation-reduction reaction generating reactive oxygen species (ROS), such as hydroxyl (·OH) and superoxide radicals (O_2^-), on their surface [8]. Using this oxidation-reduction reaction, photocatalysts kill microorganisms, such as bacteria and fungi, and inactivate viruses such as influenza virus, hepatitis C virus, vesicular stomatitis virus, enterovirus, herpes virus, Zika virus, human coronavirus, bovine coronavirus, human norovirus, murine norovirus, SARS coronavirus, and bacteriophage [8–15]. Many compounds such as titanium dioxide (TiO_2), tungsten trioxide (WO_3), zinc oxide (ZnO), cadmium sulfide (CdS), and iron (III) oxide (Fe_2O_3) are known to exhibit photocatalysis and are being actively researched. In particular, TiO_2 and WO_3 have been reported to inactivate SARS-CoV-2, and are very promising as antiviral materials [3,16,17]. In addition, photocatalysts damage the viral morphology, RNA and protein leading to the inactivation of SARS-CoV-2 [3,17]. Therefore, it is expected that photocatalysts can inactivate SARS-CoV-2, regardless of the rapidly evolving variants.

On the other hand, photocatalysts have three limitations: First, the photocatalytic reaction occurs only on the surface of the photocatalyst. Therefore, it is necessary to coat all the surfaces to avoid contamination, or to use it together with a circulator as air purifier. Second, the wavelength of light that can be used to excite the photocatalysts is limited. The wide bandgap (larger than 3 eV) of TiO_2 which is the most common photocatalyst limits the wavelength of the excitation light to the UV region [18]. Thus, narrowing the band gap of TiO_2 is very important for using the TiO_2 photocatalyst under visible light [19]. For example, mixing TiO_2 with silicane (SiH) narrows the band gap (2.082eV) and it can be excited with visible light [20]. Third, since light is necessary for the excitation of photocatalysts, the photocatalytic reaction does not occur in dark conditions, such as while sleeping.

In this study, to overcome these limitations, we used a WO_3 photocatalyst containing copper that can applied to a surface by spraying. WO_3 coating kept the surface clear of viral contamination. In addition, unlike TiO_2, WO_3 could be excited by room light even without mixing with any other compounds such as SiH. Therefore, a light source with specific wavelength was not required. Furthermore, mixing copper with WO_3 particles is expected enable the photocatalyst to inactivate the virus even in the dark, due to the effect of copper. However, there is only one study that reported the inactivation of the SARS-CoV-2 Pango lineage A by WO_3 photocatalyst [17]. In this study, we investigated the SARS-CoV-2 inactivation ability of WO_3 photocatalyst both in white light (irradiated by an light emitting diode (LED)) and in darkness, confirmed the time- and concentration-dependency of SARS-CoV-2 inactivation by WO_3 photocatalyst, and analyzed the effectiveness of this photocatalyst against different variants of SARS-CoV-2, according to Japanese Industrial Standards (JIS).

2. Results

2.1. Inactivation of SARS-CoV-2 WK-521 strain by WO_3 photocatalyst.

First, to confirm the inactivation ability of WO_3 photocatalyst against SARS-CoV-2, the WK-521 strain was placed on the WO_3 coated glass and irradiated with 1000 lx light (Figure 1A). As shown in Figure 1B, the titer of SARS-CoV-2 WK-521 strain placed on WO_3 coated glass significantly decreased after irradiation with light for 240 min, compared to before irradiation. In addition, the infectivity of SARS-CoV-2 WK-521 strain placed on WO_3 coated glass in a dark place also decreased. However, this decrease was not to the extent observed in the illuminated sample. Indeed, the mean antiviral activity values were 3.04 and 1.50 in with and without light conditions respectively (Figure 1C). This decrement in the dark condition might be due to the antiviral effect of the copper contained in the WO_3 photocatalyst. On the other hand, no decrease in the titer was observed in the samples placed on the glass without WO_3 coating for 240 min, with or without exposure to light. These results showed that the excitation light itself had no antiviral effect, and the decrease in the titer was due to the effect of the LED-WO_3 photocatalytic reaction in light, and due to the antiviral effect of copper, in darkness.

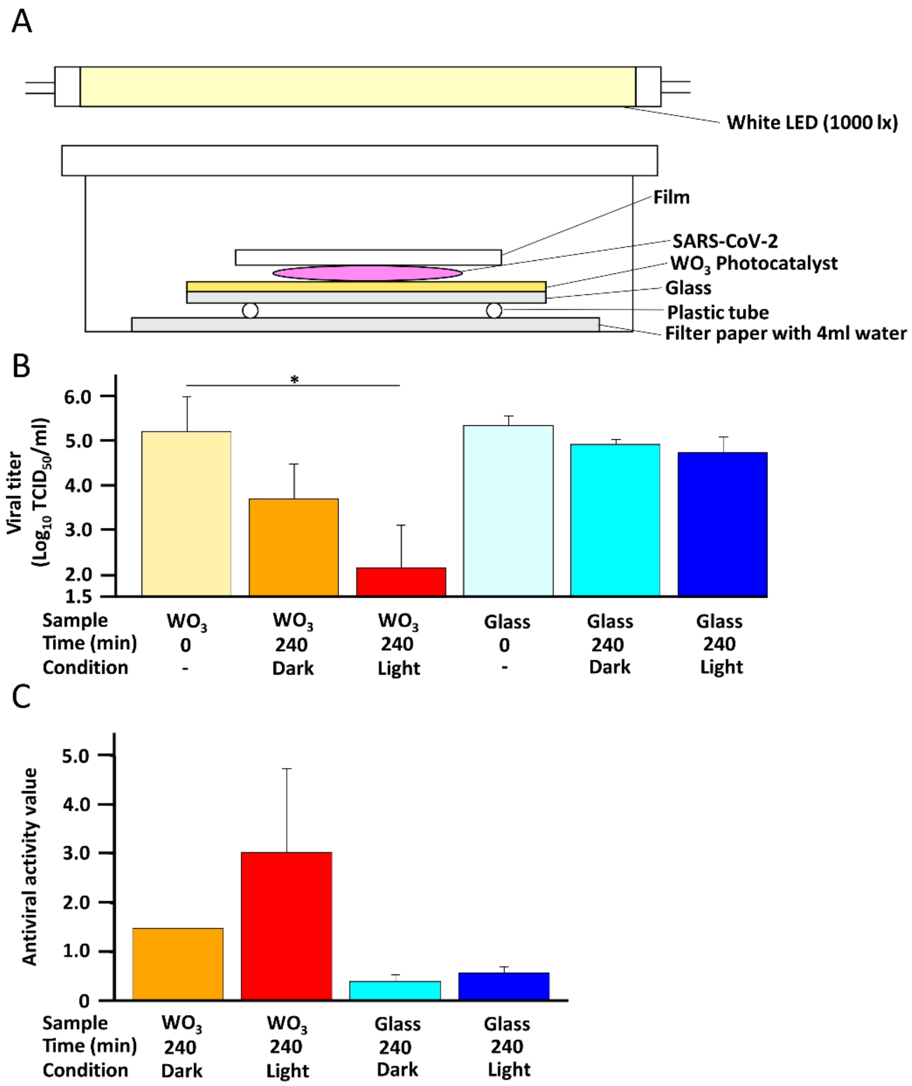

Figure 1. Inactivation of SARS-CoV-2 WK-521 strain by WO_3 photocatalyst. (A) A wet filter paper was placed in a 10 cm dish to avoid dryness. Glass with or without a coating of WO_3 photocatalyst containing copper (100 mg) was placed on a plastic tube which was in turn placed on the filter paper to avoid direct contact with the filter paper. SARS-CoV-2 WK-521 strain (150 μL) with a titer of 1×10^6 50% tissue culture infective dose ($TCID_{50}$)/mL was placed on the coated or uncoated glass. WO_3 photocatalyst was excited by white LED light with 1000 lx for 0 or 240 min. To confirm the effect of WO_3 photocatalyst containing copper in dark condition, SARS-CoV-2 WK-521 strain was placed on the glass with or without a coating of the photocatalyst, and kept in dark for 240 min. (B) Titers of SARS-CoV-2 WK-521 strain were measured using the $TCID_{50}$ assay with Vero E6/TMPRSS2 cells. Assays were performed in at least 6 wells and the values represent the mean ± standard deviation (SD) of two independent experiments. Statistical comparisons were performed using Student's t-test. Asterisk indicates a statistically significant difference (* $p < 0.05$). (C) Antiviral activity value was calculated using the formula: (The $\log_{10}$ titer of SARS-CoV-2 WK-521 strain of 240min sample) - (The $\log_{10}$ titer of SARS-CoV-2 WK-521 strain of 0 min sample of same sample).

2.2. Time- and dose-dependency of the antiviral effects of WO_3 photocatalysts.

Next, to confirm the time-dependence of the antiviral effect of the WO_3 photocatalyst, SARS-CoV-2 WK-521 strain was placed on WO_3 coated glass and irradiated with light for 0, 60, 120 and 240 min. As shown in Figure 2A, the viral titer decreased in a time-dependent manner, and the mean antiviral activity values were 0.66, 1.08 and 2.25 for 60-, 120- and 240-min light exposures respectively (Figure 2B). In addition, to confirm the antiviral effect of the WO_3 photocatalyst, SARS-CoV-2 WK-521 strain was placed on glasses coated with 10, 30 or 100 mg of WO_3 and irradiated with LED light for 0 or 240 min. As shown in Figure 2C, in the group irradiated with light for 240 min, a decrease in the titer was observed in all concentrations of WO_3 photocatalyst, compared to the group not exposed to light (0 min), which was significant in the 30 and 100 mg coatings. The antiviral activity values were 2.00, 1.25 and 0.83 for 100, 30 and 10 mg of WO_3, respectively, indicating that the titer of SARS-CoV-2 WK-521 strain was decreased by WO_3 photocatalyst in a dose-dependent manner (Figure 2D). In contrast, there was no difference in viral titers among various concentrations on WO_3 coated glass in the group which was not irradiated by LED light (0 min, Figure 2C). Our results demonstrated that photocatalysis is the mechanism of inactivation of SARS-CoV-2 WK-521 strain by WO_3, photocatalytic inactivation of SARS-CoV-2 WK-521 strain by WO_3 was dose and time dependent.

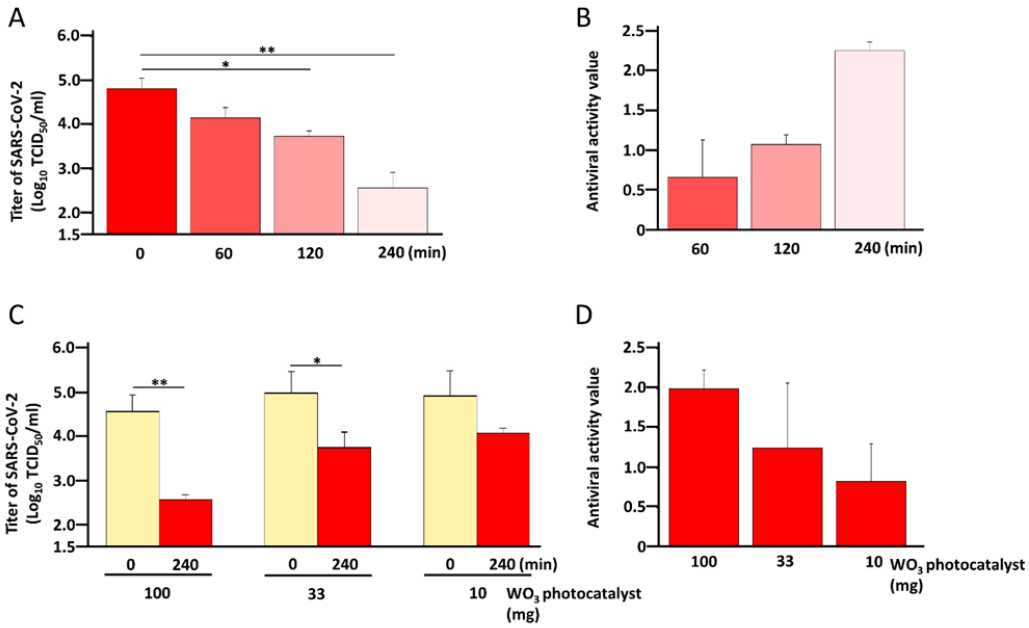

Figure 2. Time- and dose-dependency of the antiviral effects of WO_3 photocatalysts. (A) To confirm time-dependency, SARS-CoV-2 WK-521 strain (150 μL) with a titer of 1×10^6 50% tissue culture infective dose ($TCID_{50}$)/mL, were placed on glass coated with WO_3 photocatalyst (100 mg). WO_3 photocatalyst was excited by white LED light with 1000 lx for 0, 60, 120 or 240 min. The titer of SARS-CoV-2 WK-521 strain was measured using the $TCID_{50}$ assay with Vero E6/TMPRSS2 cells. Assays were performed in at least 6 wells and the values represent the mean ± standard deviation (SD) of two independent experiments. (B) Antiviral activity value was calculated using the formula: (The log_{10} titer of SARS-CoV-2 of each time point sample) - (The log_{10} titer of SARS-CoV-2 of 0min sample of same sample). (C) To confirm concentration-dependency, SARS-CoV-2 WK-521 strain (150 μL) with a titer of 1×10^6 $TCID_{50}$/mL were placed on glass coated with 10, 30 or 100 mg WO_3 photocatalyst. WO_3 photocatalyst was excited by white LED light with 1000 lx for 0 or 240 min. Titers of SARS-CoV-2 were measured using the $TCID_{50}$ assay with Vero E6/TMPRSS2 cells. Assays were performed in at least 6 wells and the values represent the mean ±SD of two independent experiments. (D) Antiviral activity value was calculated using the formula: (The log_{10} titer of SARS-CoV-2 WK-521 strain of 240min sample) - (The log_{10} titer of SARS-CoV-2 WK-521 strain of 0min sample of same concentration sample). Statistical comparisons were performed using Student's t-test. Asterisk indicates a statistically significant difference (* $p < 0.05$; ** $p < 0.01$).

2.3. WO_3 photocatalysts inactivates SARS-CoV-2 Omicron variant BA. 2.

Finally, we clarified whether the WO_3 photocatalyst exerted antiviral effect against SARS-CoV-2 Omicron variant BA. 2. The Omicron variant BA. 2 was placed on a WO_3 coated glass (150 μL with a titer of 1×10^7 50% tissue culture infective dose ($TCID_{50}$)/mL and irradiated with LED light for 0 to 240 min. As shown in Figure 3A, exposure to light for 240 min reduced the titer of this variant, similar to what was observed in the WK-521 strain. In addition, the mean antiviral activity after 240 min of photocatalytic reaction on WO_3 was 3.17, which was comparable to that observed in the WK-521 strain (Figure 3B). This result indicated that the WO_3 photocatalyst inactivates SARS-CoV-2 regardless of the variant.

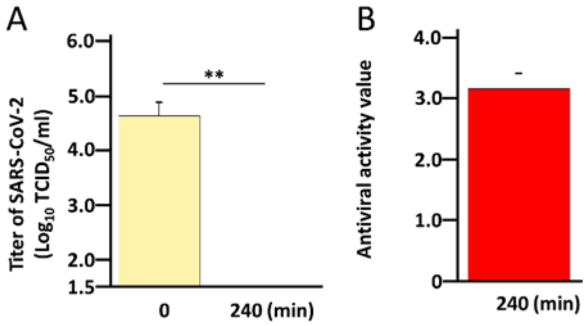

Figure 3. WO_3 photocatalysts inactivates SARS-CoV-2 Omicron variant BA. 2. (A) SARS-CoV-2 Omicron variant BA. 2 (150 μL) with a titer of 1×10^7 50% tissue culture infective dose ($TCID_{50}$)/mL was placed on glass coated with WO_3 photocatalyst (100 mg). WO_3 photocatalyst was excited using a white LED light with 1000 lx for 0 or 240 min. The titer of SARS-CoV-2 was measured using the $TCID_{50}$ assay with Vero E6/TMPRSS2 cells. Assays were performed in at least 6 wells and the values represent the mean ± standard deviation (SD) of two independent experiments. (B) Antiviral activity value was calculated using the formula: (The $\log_{10}$ titer of SARS-CoV-2 of 240 min sample) - (The $\log_{10}$ titer of SARS-CoV-2 of 0 min sample). Statistical comparisons were performed using Student's t-test. Asterisk indicates a statistically significant difference (** $p < 0.01$).

3. Discussion

In this study, we demonstrated that the WO_3 photocatalyst containing copper effectively inactivated SARS-CoV-2. Indeed, our results provide evidence that WO_3 photocatalytic reaction for 240 min significantly decreased the infectivity of SARS-CoV-2 WK-521 strain. Additionally, the copper present in the photocatalyst enabled it to inactivate the virus even in darkness. Furthermore, the WO_3 photocatalyst containing copper decreased the SARS-CoV-2 WK-521 strain titers in a time-and dose-dependent manner, confirming the photocatalysis induced inactivation of the virus. Our results are supported by a previous report that showed the effective inactivation of SARS-CoV-2 by a WO_3 – based visible light-responsive photocatalyst on under different temperatures and exposure durations [17]. Notably, we demonstrated the effectiveness of a 240 min photocatalytic reaction involving WO_3 photocatalyst containing copper, not only against the SARS-CoV-2 WK-521 strain but also against the Omicron variant BA. 2, as indicated by the decreased viral titers. The present study is the first to report that a WO_3 photocatalytic reaction can inactivate SARS-CoV-2, regardless of the variant.

It is previously reported that the mechanisms involved in the inactivation of SARS-CoV-2 by photocatalysis are damage of viral morphology, RNA, and protein [3,17]. In this study, the inactivation of the SARS-CoV-2 WK-521 strain and the Omicron variant BA. 2 by WO_3 photocatalyst containing copper was demonstrated. This suggests that even if the virus is mutated, a photocatalytic reaction by WO_3 can achieve viral inactivation by damaging the viral protein, RNA and lipid bilayer, irrespective of the variant. Hence, this WO_3 photocatalyst containing copper, could be effective against the variants of SARS-CoV-2 which may in the future.

WO_3 photocatalyst containing copper inactivated SARS-CoV-2 not only upon irradiation with light, but also in dark condition as well. It has been reported previously that copper nanoparticles can inactivate SARS-CoV-2 [21]. In addition, copper oxide (CuO) nanoclusters grafted with titanium dioxide also inactivated SARS-CoV-2 alfa, beta, gamma and delta variants under illumination and in dark conditions as well [22]. These observations suggest that copper is responsible for the inactivation SARS-CoV-2 WK-521 strain under dark condition, observed in this study, indicating that the WO_3 photocatalyst containing copper can be effective even in the night time.

Since WO_3 photocatalyst containing copper was excited by a white LED in this study, it is evident that this photocatalyst works effectively in indoor environment, without the necessity for any specific light source. In addition, unlike UV, WO_3 photocatalyst is harmless to the human body. Therefore, it is expected that WO_3 photocatalyst containing copper can be used for disinfection on surfaces which are touched regularly by many people, thereby preventing the viral spread. Thus, this study demonstrated that WO_3 photocatalyst containing copper could be effectively applied to control SARS-CoV-2 transmission and mitigate the ongoing COVID-19 pandemic.

4. Materials and Methods

4.1. Virus and cell culture

Vero E6 cells which express the transmembrane serine protease TMPRSS2 (Vero E6/TMPRSS2 (JCRB1819)) were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Thermo Fisher Scientific) at 37 °C with 5% CO_2. The WK-521 strain (Pango lineage A; 2019-nCoV/Japan/TY/WK-521/2020) [23] and Omicron variant BA. 2 (hCoV-19/JPN/TY40-385/2022 strain) of SARS-CoV-2 were cultured and quantified using Vero E6/TMPRSS2 cells in DMEM containing 2% FBS. The infectivity of the viral strains was calculated by titrating in Vero E6/TMPRSS2 cells using the $TCID_{50}$ assay and applying the Reed-Muench method [24].

4.2. Inactivation of SARS-CoV-2 by WO_3 Photocatalytic Reaction

The photocatalytic reaction was performed according to JIS R1752:2020 [25] with a minor modification (Figure 1A). Briefly, filter paper was placed at the bottom of the 10 cm dish and got wet by 4 mL sterilized water for moisture preservation. To avoid directly touching the filter paper, a plastic tube was placed on the filter paper and a glass coated with 100 mg WO_3 photocatalyst containing copper (NFE2-W; Chemical Technology Co., Ltd., Takaishi, Osaka, Japan) was placed on top of the plastic tube. 150 µL of the WK-521 strain with a titer of 1×10^6 $TCID_{50}$/mL or the Omicron variant BA. 2 with a titer of 1×10^7 $TCID_{50}$/mL was placed on the WO_3 coated glass and spread by covering it with a film. Glass without WO_3 coating was used as a negative control. The samples were then illuminated with 1000 lx light using a white LED (BBZ T13 Silver; Dongguan oushi Electronic Technology Co., Ltd, Dongguan, Guangdong China) for 240 min, or not illuminated. After illumination, the samples were washed by immersing in 8 mL phosphate buffered saline (PBS). As time control, the virus was immediately collected after placing the droplet on glass with or without WO_3 coating (0 min). To confirm the time-dependency, the SARS-CoV-2 WK-521 strain was placed on the 100 mg WO_3 coated glass and illuminated for 0, 60 ,120 and 240 min. To observe the concentration-dependency, the SARS-CoV-2 WK-521 strain was placed on glass coated with 100, 30 or 10mg WO_3 and illuminated for 0- and 240-min. SARS-CoV-2 titers in all experiments was measured by $TCID_{50}$ assay.

The photocatalytic inactivation efficiency was defined as follows:

$$\text{Antiviral activity value} = \log_{10}(N_t) - \log_{10}(N_0)$$

where N_t represents the virus titer of the photocatalytically treated specimens after irradiation for t hours; N_0 represents the virus titer of the photocatalytically treated specimens just after inoculation (0 min).

4.3. Statistical analysis

Statistical comparisons were performed using Student's t-test. p values < 0.05 were considered statistically significant.

5. Conclusions

This is the first report showing that WO_3 photocatalyst inactivates Omicron variant BA. 2 as well as SARS-CoV-2 WK-521 strain, indicating the effectiveness of this photocatalyst against the virus, regardless of the variant. In addition, WO_3 photocatalyst containing copper can inactivate the virus using a simple white light or even in dark conditions, indicating its potential for wide application. In conclusion, WO_3 photocatalyst containing copper could be a very effective tool for controlling the spread of SARS-CoV-2.

Author Contributions: Conceived and designed the experiments: Y.A., and R.M. Conducted and performed the experiments: K.M, R.M., and Y.A. Analyzed the data: R.M., and Y.A. Supervised this experiment: Y.A., K.M, K.H, Y.M. (Yosuke Mori) and Y.M. (Yasunobu Matsumoto). Contributed reagents/materials/analysis tools: Y.A., K.M., K.H, Y.M. (Yosuke Mori), and T.K. Wrote the paper: R.M., and Y.A. All authors have read and agreed to the published version of the manuscript.

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