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# Photocatalytic Disinfection of *Legionella pneumophila* on Silver-Doped Titania Thin Films

Eiko Obuchi[1], Keisuke Yanagi[1], Katsumi Katoh[1], Ataru Kuroiwa[2], Toshinori Soejima[2] & Katsuyuki Nakano[1]

[1] Department of Chemical Engineering, Fukuoka University, Fukuoka, Japan

[2] Department of Microbiology and Immunology, School of Medicine, Fukuoka University, Fukuoka, Japan

Correspondence: Eiko Obuchi, Department of Chemical Engineering, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. Tel: 81-92-871-6631. E-mail: eobuchi@fukuoka-u.ac.jp

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**Abstract**

The activities of thin films of titania and silver-doped titania for the photocatalytic disinfection of *Legionella pneumophila* in aqueous medium were examined and compared with each other. Both photocatalytic films disinfected *L. pneumophila* under UV irradiation; the Ag-doped film showed a higher activity than the non-doped one. The silver-doped titania film even showed a slight activity in disinfecting *L. pneumophila* under the absence of UV irradiation. This bactericidal effect in darkness overcomes a weakness of photocatalytic disinfection. Scanning electron micrographs of *L. pneumophila* after photocatalytic disinfection showed that the cell walls of the bacteria were destroyed in the presence of the thin film of Ag-doped titania. Furthermore, the photocatalytic action caused decomposition of the endotoxin leaked from the dead bacteria.

**Keywords:** silver, titania, disinfection, *Legionella*, photocatalysis

## 1. Introduction

Many bacteria that are normally present in our surroundings can cause serious disease in the elderly, the infirm, infants or other persons with compromised immune systems. *Legionella pneumophila* is well-known bacterium that can cause serious pneumonia (legionnaires' disease). In Japan, these bacteria are occasionally detected in facilities such as hot-spring resorts or cooling towers. In general, water in such facilities is disinfected by treatment with chlorine. However, because *L. pneumophila* is tolerant to chlorine, it can still cause infection. Therefore, a new method of sterilization using ultraviolet (UV) radiation or ozone is required instead of chlorination. Sterilization in the presence of a photocatalyst is one possible method of sterilization that has attracted some attention.

Titanium dioxide ($TiO_2$, titania) is the most widely used photocatalyst; titania is safe enough to add to food, cheap, and stable. When it is subjected to UV irradiation (under 380 nm), titania generates hydroxyl radicals that are strong oxidizing agents capable of fully decomposing organic compounds to $CO_2$ and $H_2O$. In early stage of research using $TiO_2$ photocatalyst, many researchers used $TiO_2$ powders (Augugliaro et al., 1991; Hoffmann, Martin, Choi, & Bahnemann, 1995). For practical purposes, however, it will be necessary to immobilize the photocatalyst (Chen & Dionysiou, 2006; R. Nakano, Chand, Obuchi, Katoh, & K. Nanano, 2011). Furthermore, researches were also expanded which various metals were doped on $TiO_2$ in order to add a new function, such as a visible light response (Chand, Obuchi, Katoh, Luitel, & Nakano, 2011; Liu, P. Wang, X. Wang, H. Yu, & J. Yu, 2012).

On the other hand, because bacteria consist of organic compounds such as proteins, it is possible to decompose them by using a suitable photocatalyst (Rincón & Pulbarin, 2003; Reddy, Venugopal, & Subramanyam, 2007; Rengifo-Herrera et al., 2008). In the field of bacteria disinfection, metal doped $TiO_2$, such as Ag-doped $TiO_2$ (L. Liu, Z. Liu, Bai, & Sun, 2012; Ubonchonlalate, Sikong, & Saito, 2012) has been studied. As it is said from ancient times that silver has a bactericidal effect, so that Ag-doped $TiO_2$ has attracted having higher activity for disinfection than non-doped $TiO_2$ photocatalyst. In a previous study (Matsuo, Takeshita, & Nakano, 1990; Obuchi, Yamamoto, & Nakano, 1995; Obuchi, Hashimura, & Nakano, 1999), we developed a new method for preparing transparent thin films of titania that showed photocatalytic activity against various organic compounds, such as 2, 4-dinitrophenol, under UV irradiation (Obuchi et al., 1995; Shiraishi, Toyoda, Fukinbara, Obuchi, &

Nakano, 1999). We have also tested the ability of these films to inactivate viruses by using *Lactobacillus* PL-1 phages (Kakita et al., 2000) and the ability of titania thin film coated on glass plates to disinfect *L. pneumophila* (Hayakawa, Kuroiwa, Higashi, & Nakano, 2007). In this work, transparent Ag-$TiO_2$ solution which doped Ag to the transparent titania developed in the previous report (Obuchi et al., 1995) was prepared. We report on our investigations of the comparative activities of titania and Ag-doped titania thin film coated on glass tube in the photocatalytic disinfection of *L. pneumophila* in aqueous medium.

## 2. Material and Methods

### *2.1 Preparation of the Photocatalysts*

Amorphous titania powder, which prepared by hydrolysis and condensation polymerization of titanium tetra-iso-propoxide (Kishida Chemical Co. Ltd., Osaka), as reported previously (Obuchi et al., 1995), was dissolved in 30% aqueous hydrogen peroxide (Wako Pure Chemical Industries, Ltd., Tokyo). In the case of the Ag-doped titania, the required quantity of silver acetate (Wako Pure Chemical Industries, Ltd., Tokyo) which consist of 3 wt% to titania powder was added at the same time, and the mixture was stirred at 293 K for 2 hours. A transparent yellow gel was formed with the bubble of oxygen generated by decomposition of hydrogen peroxide. The gel was peptized again by adding 30% hydrogen peroxide aqueous solution and then the yellow transparent solution was stirred until generating of oxygen was completed. Thus, transparent titania or silver doped titania solution were obtained.

The interior of a cleaned quartz glass tube (internal diameter: 10 mm, outside diameter: 12 mm, length: 270 mm) was coated with the titania or Ag-doped titania solution. The coated tube was dried at room temperature, then calcined at 773 K for 2 hours. These operations (coating - drying - calcination) were repeated 10 times. Thus, the glass tube reactors coated with a thin film of titania or Ag-doped titania were obtained. About 300 nm of thickness of the titania thin films were also prepared using the same titania solution on glass plates calcined at 473-773 K for X-ray diffraction (XRD) analysis. XRD patterns of titania thin film were measured using Mac science M03X-HF (Model No. 1031) with Cu Kα irradiation ($\lambda$ = 1.54050 Å) in the range of 20-60 ($2\theta$), operated at 40 kV and 20 mA.

### *2.2 Experimental Procedures*

The strain of *L. pneumophila* (Serogroup1) was donated by Professor Yoshida (Department of Microbiology, Faculty of Medical Science, Kyushu University). This strain was cultured on buffered charcoal yeast extract (BCYE) agar plates (Eiken Chemical Co. Ltd., Tokyo) at 310K for at least seven days. The bacteria were then suspended in sterile water and adjusted to $10^5$cfu/mL with sterile water to prepare the test suspension.

Figure 1 is a schematic of the experimental apparatus that we used in this work. A 200 mL portion of the test suspension of *L. pneumophila* was filled into the titania-coated or Ag-doped titania-coated glass tube and circulated at a flow rate of 200 mL/min. Black-light lamps (BL, 6W ×4, wavelength: 300 - 400 nm, UV intensity: 5.0 mW/cm$^2$) or germicidal lights (GL, 6 W ×4, wavelength: 254 nm, UV intensity: 7.0 mW/cm$^2$) located outside the glass tube were switched on to illuminate UV light. Small aliquots were then withdrawn from the suspension at intervals and examined for the presence of viable bacteria. The sampled suspensions were cultured on BCYE agar plates at 310 K for seven to ten days to determine the viable cell count ($N$). The survival rate at each sampling time was defined as the ratio of $N$ to the initial viable cell count ($N_0$) and is reported as a percentage. The experiments were repeated three times with different cultures of *L. pneumophila*, and reproducibility in data was observed.

The photocatalytic disinfection of *L. pneumophila* in Ag-doped titania-coated reactor was performed under the irradiation with the germicidal light for 24 hours. The test solution of 2 mL, was sampled at time intervals (0, 0.25, 0.5, 1, 4, 8, 12, and 24 hours), and endotoxin concentration was measured by Limulus Amoebocyte Lysate assay (lower limit of detection: 1.0 pg/mL).

A scanning electron microscope (JSM-6060; JEOL Ltd., Tokyo) was used to observe the shapes of the *L. pneumophila* before and after photocatalytic treatment for 12 hours. The samples for SEM observation were prepared as in our previous study (Hayakawa et al., 2007).

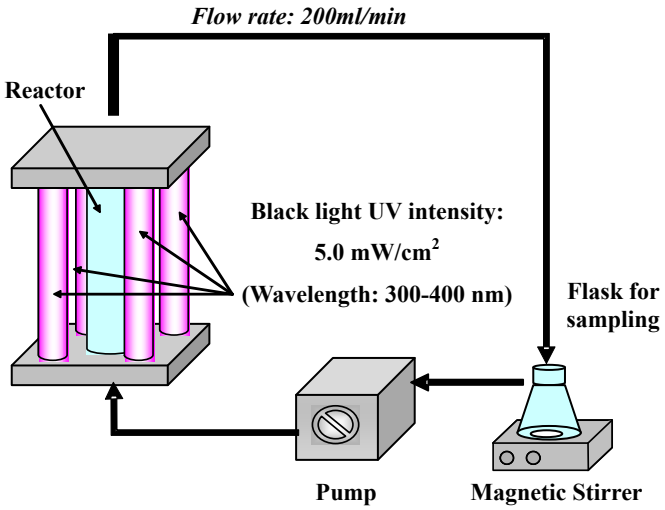


Figure 1. Schematic of the experimental apparatus

## 3. Results and Discussion

### *3.1 XRD Analysis of Titania Thin Films*

Figure 2 shows the XRD patterns of titania thin films calcined at 473-773 K. Since anatase (101) peak (2θ = 25.3°) in the titania thin film calcinated at 473 K is identified, it is found that crystallization to anatase started at low temperature. Other XRD patterns of titania thin films were clarified to be anatase phase of peaks relating to (101), (004), (200), (105), and (211) at 25.3, 37.8, 48.1, 53.9 and 55.1°, respectively (Kiyono, 1991). Unfortunately, however, no peak of Ag was observed in the Ag-doped titania thin films. It is supposed because of the quantity of the doped silver, it is only 3wt% to titania. Other features of titania thin films, for example, UV-vis spectra and SEM image, were reported in previous papers (Matsuo et al., 1990; Obuchi et al., 1995; Obuchi et al., 1999).

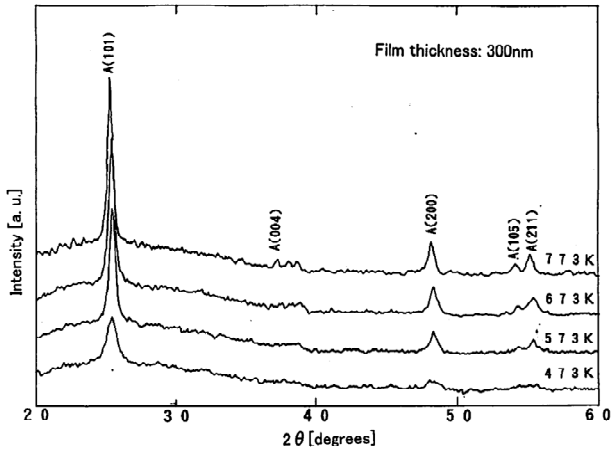


Figure 2. XRD patterns of titania thin films on the glass plate calcined at 473-773 K

### *3.2 Photocatalytic Disinfection in Titania-Coated and Ag-Doped Titania-Coated Reactors*

Figure 3 shows a plot of the survival rate of *L. pneumophila* against the process time for reactors coated with the thin film of titania (P-Ti) or Ag-doped titania (Ag-Ti) under black-light UV irradiation. The results follow a first-order rate law, and the estimated rate constants ($min^{-1}$) are shown in the figure. In the absence of a photocatalyst and with no UV irradiation (control), there was no disinfection of *L. pneumophila*. On the other hand, under UV irradiation, the survival rate of *L. pneumophila* decreased gradually in the absence of a

photocatalyst. In the presence of a thin film of titania or Ag-doped titania under UV irradiation, the survival rate of *L. pneumophila* decreased rapidly, showing that the thin films of titania and Ag-doped titania that we prepared have photocatalytic activities. Surprisingly, however, the Ag-doped titania film also showed a slight disinfection of *L. pneumophila* in the absence of UV irradiation. Although it has been claimed that silver has a general bactericidal effect, the mechanism by which this effect is produced is unclear. However, our results suggest the possibility that the presence of Ag as a dopant might serve to overcome one of the weaknesses of photocatalytic disinfection, its inability to operate in darkness.

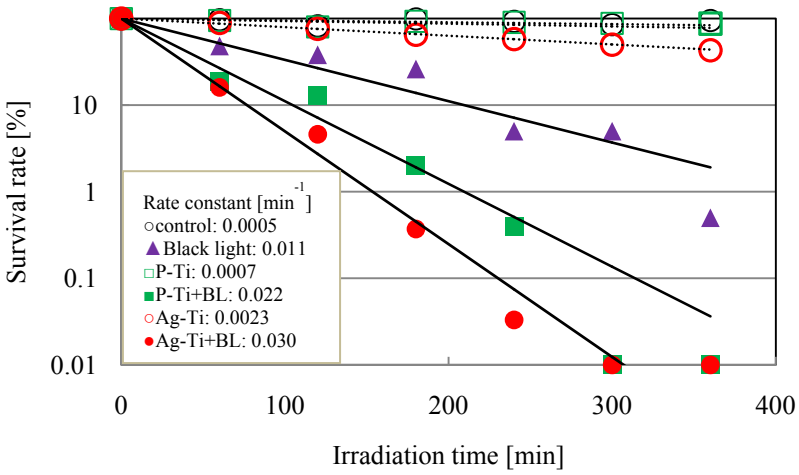


Figure 3. Plot of the survival rate of *L. pneumophila* against the process time. BL: black light; P-Ti titania thin film; Ag-Ti: silver-doped titania thin film

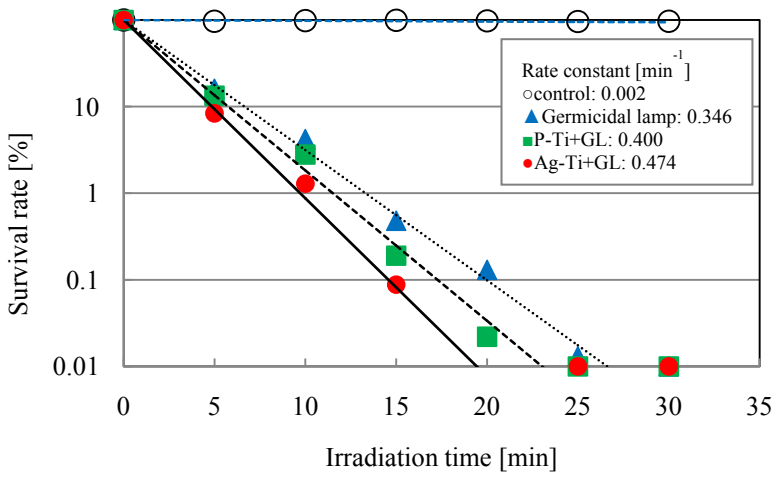


Figure 4. Plot of the survival rate of *L. pneumophila* against the process time for Ag-Ti and P-Ti thin film with irradiation by the germicidal light. GL: germicidal light; P-Ti titania thin film; Ag-Ti: silver-doped titania thin film

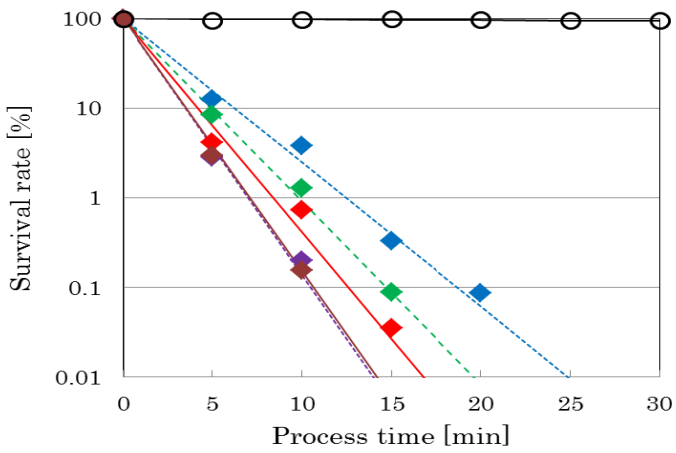


Figure 5. Plot of the survival rate of *L. pneumophila* against the process time at various flow rates in the presence of thin films of silver-doped titania films and GL irradiation

○: Control; ◆: Ag-Ti + GL (◆: 100; ◆: 200; ◆: 600; ◆: 800; ◆: 1000 mL/min)

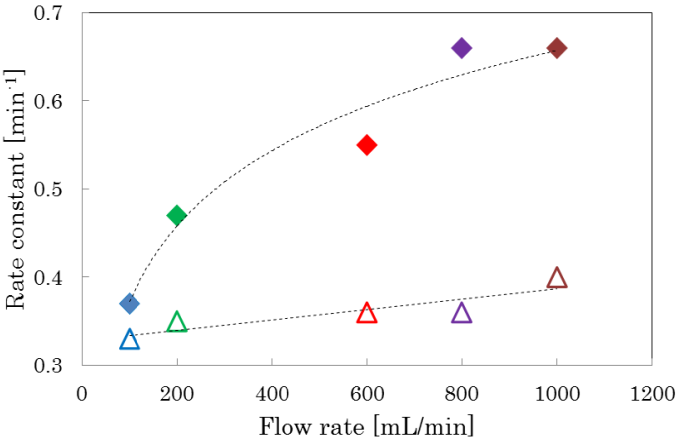


Figure 6. Dependence of the reaction rate constant on the flow rate

△: GL (△: 100; △: 200; △: 600; △: 800; △: 1000 mL/min)

◆: Ag-Ti + GL (◆: 100; ◆: 200; ◆: 600; ◆: 800; ◆: 1000 mL/min)

As shown in Figure 4, when the germicidal light (GL) was used as a source of UV radiation instead of the black light (BL), the survival rate of *L. pneumophila* decreased markedly regardless of the presence or absence of the photocatalyst. Because the germicidal light produces sterilizing rays at around 254 nm, it disinfects *L. pneumophila* by its own action rather than through activation of a photocatalyst. When thin films of titania or Ag-doped titania were used under GL irradiation, the disinfection rates were 15-18 times higher than those under BL irradiation. In our previous paper (Hayakawa et al., 2007), we reported *L. pneumophila* could be disinfected photocatalytically using titania thin film on a glass plate irradiated with UV. Yao, Ochiai, Ishiguro, Nakano and Kubota (2011) reported photocatalytic disinfection of *L. pneumophila* using $TiO_2$-coated ceramic foam and $Cu^{2+}/TiO_2$-coated ceramic foam in batch reactor (Ishiguro et al., 2013). In this work, it was found that *L. pneumophila* could be disinfected while circulating the test solution using the reactor coated with titania or Ag-doped titania thin films, and the germicidal light (wavelength: 254 nm) is more effective compared with BL for practical processing, where continuous sterilization system is utilized.

Figure 5 shows the changes in the survival rates of *L. pneumophila* when the flow rate of the test suspension was increased in stages from100 ml/min to 1000 ml/min under GL irradiation in the presence of Ag-doped titania. The rate constants for *L. pneumophila* disinfection increased almost proportionately with increasing flow rate

(Figure 6). In the case of a low flow rate, a thick boundary layer is present between the surface of the photocatalytic thin film and the test suspension containing *L. pneumophila*, whereas at higher flow rates, this boundary layer becomes thinner. The rate of mass transfer of *L. pneumophila* to active sites on the surface of the photocatalyst is dependent on the thickness of the boundary layer. Therefore, in the case of a high flow rate, *L. pneumophila* can move easily to the active sites, and the rate constant for *L. pneumophila* disinfection increases. When GL irradiation was used alone without a photocatalyst, the flow rate of the test suspension had barely any effect.

*3.3 Scanning Electron Microscopy Observations of L. pneumophila*

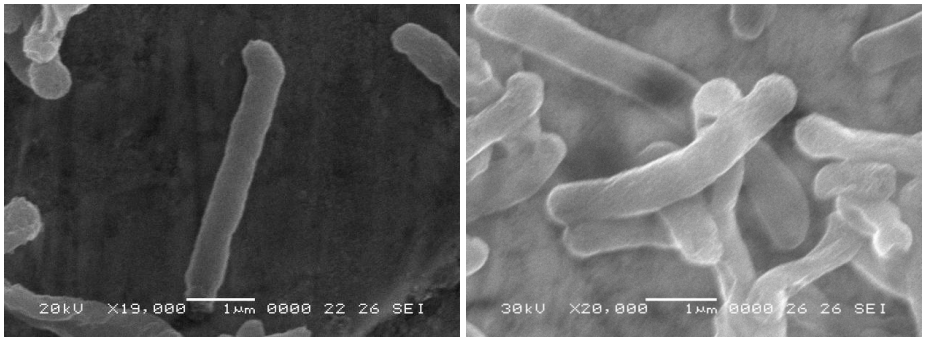


Figure 7(a). Scanning electron micrographs of *L. pneumophila* (Control)

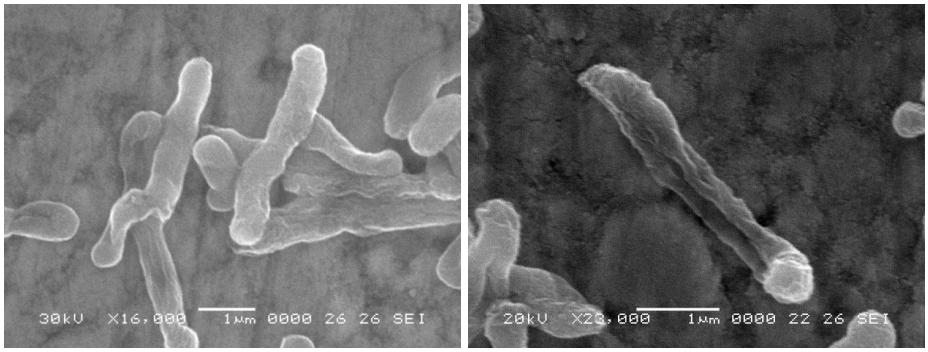


Figure 7(b). Scanning electron micrographs of *L. pneumophila* irradiated with the GL

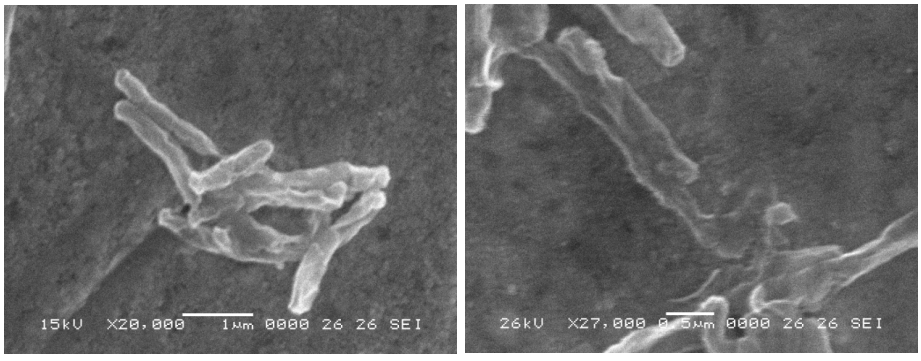


Figure 7(c). Scanning electron micrographs of *L. pneumophila* irradiated with the GL in the presence of a thin film of titania (P-Ti)

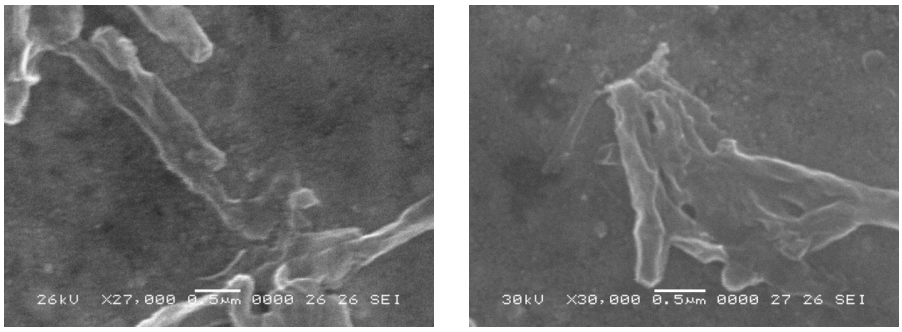


Figure 7(d). Scanning electron micrographs of *L. pneumophila* irradiated with the GL in the presence of a thin film of silver-doped titania (Ag-Ti)

Scanning electron micrographs of *L. pneumophila* after various treatments for 12 hours are shown in Figures 7(a) - 7(d). Figure 7(a) is of the control. Figure 7(b) shows the bacteria after GL irradiation in the absence of a photocatalyst. Figures 7(c) and 7(d) show bacteria subjected to GL irradiation in the presence of thin films of titania (P-Ti) or Ag-doped titania (Ag-Ti), respectively. When the germicidal light was used as the radiation source, the survival rate constant of *L. pneumophila* was similar in the presence or absence of photocatalyst, as discussed in Section 3.2. However, in the scanning electron micrographs, the shapes of the *L. pneumophila* treated in the presence of the two films show obvious differences from those treated by irradiation alone. The appearance of *L. pneumophila* subjected to GL irradiation in the absence of a photocatalyst was relatively unchanged, although wrinkles developed on the surfaces of the bacteria. On the other hand, the cell walls of *L. pneumophila* were destroyed in the presence of the thin-film Ag-titania photocatalyst. This clearly shows that the photocatalyst has an additional effect to GL irradiation alone.

Endotoxin, a component of the outer membrane of Gram-negative bacteria, leaks from dead bacteria and is highly toxic, even at very low concentrations. When the outer membrane of the *L. pneumophila* was destroyed, as observed in Figure 7, a leakage of endotoxin was detected. However, the endotoxin was photocatalytically decomposed after 12 hours of treatment, as shown in Figure 8. Sunada, Kikuchi, Hashimoto, and Fujishima (1998) already reported endotoxin from *E. coli* could be decomposed using $TiO_2$ photocatalyst. In our work, it was shown clearly that endotoxin could be decomposed below the detection limit within 24 hours, despite high concentration of endotoxin (2,000 pg/mL) leaked from dead *L. pneumophila*. For removal or inactivation of endotoxin leaked from dead bacteria, the filtration using reverse osmosis and inactivation using some chemicals have been known currently in practical processes. The result from our experiments could show that endotoxin was decomposed simultaneously during the sterilization of *L. pneumophila*, without the chemical or the physical processing mentioned above.

Considering the practical utilization of photocatalyst, the durability of a thin film is very important. It had already reported that a titania thin film had tolerance to acid or alkali (Obuchi et al., 1999). In this paper, it was examined whether it could be used repeatedly and be kept photocatalytic activity of Ag-doped titania thin film for long time. Figure 9 shows the plots of the survival rate of *L. pneumophila* vs. total irradiated time. At first, photocatalytic sterilization of *L. pneumophila* was performed during 30 minutes under GL irradiation, and stopped the operation for cleaning up. In the clean-up operation, the reactor tube was heated at 60 °C and 2 atm in the autoclave for 15 minutes. After the clean-up operation, the fresh test solution including *L. pneumophila* (the concentration was approximately $6x10^5$ cfu/mL) was added. This sterilization and cleaning cycle was repeated 10 times. No viable *L. pneumophila* was detected after 25 minutes for each cycle, so that a thin film of Ag-doped titania was found to retain the photocatalytic activity.

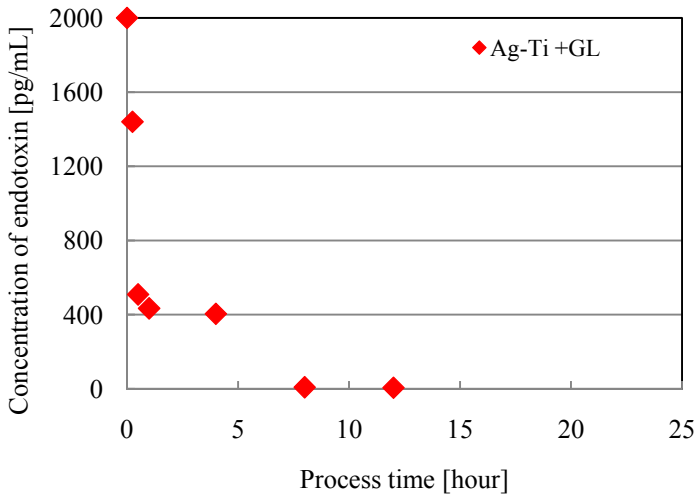


Figure 8. Plot of the concentration of endotoxin of L. pneumophila against the process time using Ag-Ti thin film with irradiation by the germicidal light

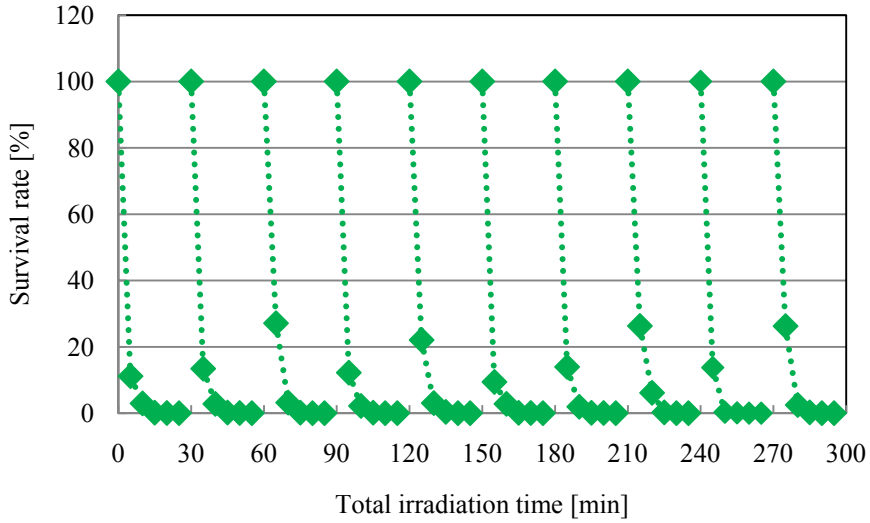


Figure 9. Continual sterilization operations using a Ag-Ti coated quartz glass tube with irradiation by the germicidal lights. Every 30 minutes, fresh test solution including *L. pneumophila* was added in the reactor

## 4. Conclusions

We conclude that thin films of titania or Ag-doped titania can disinfect *L. pneumophila* effectively when subjected to UV irradiation. Endotoxin leaking from the dead *L. pneumophila* can be decomposed simultaneously within 24 hours. Ag-doped titania shows a slight disinfecting effect on *L. pneumophila* in even in the absence of UV irradiation.

The titania and Ag-doped titania solutions prepared in this work can be readily coated onto glass, heat-resistant steel, or other materials; that might be useful in designing and developing an attractive new type of practical reactor for disinfecting various kinds of bacteria, such as *Staphylococcus aureus*, MRSA, *E. coli*, and *Bacillus subtilis*, in aqueous media (Obuchi, Fujikawa, Katoh, Kuroiwa, & Nakano, 2011).

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