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Antibacterial device using UVA-LED and TiO_2 films for air-conditioner evaporator

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Recently, photocatalysis with TiO_2 was very restricted because of the lack of an appropriate UV source. In this work, in order to increase antibacterial properties of UVA-LED, photoreactive TiO_2 film was coated on aluminum substrates by *rf*-magnetron sputtering. The number of *Escherichia coli* which remained after irradiation under each time was counted. The photocatalytic TiO_2 films on aluminum after irradiation at 365 nm have the comparable antibacterial ability to the uncoated sample irradiated at 280 nm. A sterilization system integrating TiO_2 film with UVA-LED has the potential for air purification in automobile and would find wide use in the field of antibacterial.

Key words: UVA-LED, TiO_2 film, Antibacterial ability.

Introduction

For many ultraviolet (UV) applications, there are sustainability issues that arise from current low-pressure lamp in use. Disadvantages of mercury lamps such as mercury waste products, short life span of the lamp, lost energy in the form of non-light-producing heat and high cost have led researchers to seek a new environmentally friendly technology that overcomes these disadvantages. Light emitting diodes (LEDs) may give solutions to the complicated issues of UV mercury lamps. LED is much smaller and much lighter than the low-pressure mercury lamp and do not contain glass, filament or mercury. Limited research has been performed on the effectiveness of UV-LED for UVA range. Most of the data available are for LEDs that emit light of UVC or UVB ranges (200 ~ 300 nm), which is more efficient at sterilization than light in the range of UVA (320 ~ 400 nm) [1, 2]. Recently, UVA-LEDs are commercialized and verified, while the LEDs in the wavelength range of 200 ~ 350 nm still need to improve its reliability and to reduce the price. Moreover, as far as we know, there is a few information on the difference of sterilization effects between UVA and UVC.

It was found in 1985 for the first time that TiO_2 had antibacterial properties, which were attributed to the appearance of hydroxyl radicals and superoxide radical anions on the surface species under UV irradiation. The TiO_2 (band gap = 3.2 eV) produced hole and electron pairs when exposed to UV light at about 387 nm. Although TiO_2, as a photo-induced antibacterial agent, has attracted increasing interest [3], photocatalysis with TiO_2 was very restricted because of the lack of an appropriate light source.

As described previous works [4, 5], moisture condensed on an automobile air-conditioner evaporator made by aluminum (Al) can trap dust and create an environment where microorganisms thrive. These bacteria and mold gradually form a "slime layer" of polysaccharides. Technically called a 'bio-film', this slime layer has been a focus of much current research in microbiology.

The objective of this work was to evaluate the efficiency of UV-LED for sterilization. Specifically, this work evaluated the use of UV-LEDs at 280 nm and 365 nm, respectively, for inactivation of *Escherichia coli* (*E. Coli*). All tests were completed within 60 min and irradiated specimens will be covered to minimized photo-reactivation as much as possible.

In order to increase antibacterial effect UVA-LEDs at 365 nm, UV-photoreactive TiO_2 films were coated on Al substrates. The number of bacteria which remained after irradiation under each condition was counted.

Experimental

Experimental device which contained high power UV-LEDs at 280 nm and 365 nm (Seoul Optodevice, Korea) was designed. The Al base copper clad laminates was used here as a support for the UV-LEDs. Modules were designed with an array of six LEDs using a circuit wire-wrapped for the LEDs at 280 nm and 365 nm. Two types of UV-LED irradiation protocols were used in this work. 120 Ω and 560 Ω resistors for the modules

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at 280 nm and 365 nm, respectively, were wired in series with each LED to create 6.6 V and 3.3 V across each LED at 80 mA and 40 mA with a 9 V input voltage from a power supply.

E. coli strain (DH5α) was purchased from Korean Culture Center of Microorganisms (KCCM 11234) for disinfecting index [6]. *E. coli* is used extensively as an index of contamination levels of water. Stationary phase *E. coli* cells grown on a Luria-Bertani agar plate (LB plate) were used. *E. coli* cells cultured overnight in LB broth were collected by centrifugation for 3 min at 10,000 rpm and 4 °C, washed twice with phosphate buffered saline (PBS, pH 7.4), and adjusted to an optical density (O. D.) of 1.0 at 600 nm for a bacterial concentration of 2×10^8 colony forming units (CFUs) per mL for batch irradiation testing. Then 600 μL of saline solution with bacteria was added drop wise onto the surface of each sample. After irradiation at 280 nm and 365 nm for various times, the specimens were washed using 10 ml buffer solution which was subsequently spread onto a nutrient agar plate. After spreading, all Petri dishes were incubated overnight at 37 °C, and the numbers of colonies were counted and averaged.

All tests were completed within 1 hour and irradiated samples were covered to minimize photo reactivation as much as possible. Suspension of DH5α 600 μL drops on the sample. It was irradiated with LED into acrylic box (150 mm × 150 mm × 250 mm). The distance between LED modules and bacterial suspension was 20 mm.

After irradiation, each sample was successively vortexed and the substrates were placed in a 37 °C incubator and incubated for 24 hours before colonies were counted. The number of bacteria which remained after irradiation under each condition was counted. To evaluate the sterilization effect in UV irradiation by LED, the number of bacteria after irradiating was divided by the number of before irradiation. The survival rate according to colony-forming ability assay is calculated by the equation $\{\mathbf{R}(\%) = [(N - N_0)/N] \times 100$, **R**: *Escherichia coli* reduction rate, N: Number of bacteria colonies before irradiation, N_0: Number of bacteria colonies after irradiation$\}$.

In order to increase antibacterial efficiency at 365 nm, photoreactive TiO_2 thin films were coated on Al substrates by *r. f.* magnetron sputtering. The base vacuum of sputtering chamber is 5.6×10^{-6} torr, and high purity oxygen and argon gas with pressure of 5.0×10^{-5} torr are used. The *r. f.* power is 150 W. The coatings are deposited on Al substrates for automobile case study. During the deposition, the substrates were intentionally heated at 200 °C. As-deposited films were annealed to 500 °C for 1 hr in order to convert from amorphous state to anatase TiO_2.

Results and discussion

Surface morphology of the Al substrates and TiO_2 films was evaluated by field emission-scanning electron microscope (FE-SEM, S-4700, Hitachi, Japan). Fig. 1(a) shows the FE-SEM photograph of the Al used in this study. Well-grown 0.1 μm sized crystallites were obtained. TiO_2 films present smooth dense surface, no visible pores and defects over the TiO_2 film, as shown in Fig. 1(b). It further confirms that TiO_2 films coated on Al are composed by an anatase crystalline structure without a significant amount of any other TiO_2 phase, not shown here.

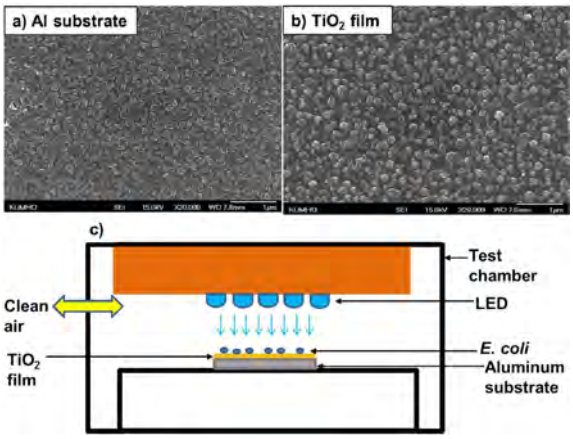

Fig. 1. FE-SEM photographs of (a) Al substrates and (b) TiO_2/Al and a diagram of platform for examination of antibacterial effect.

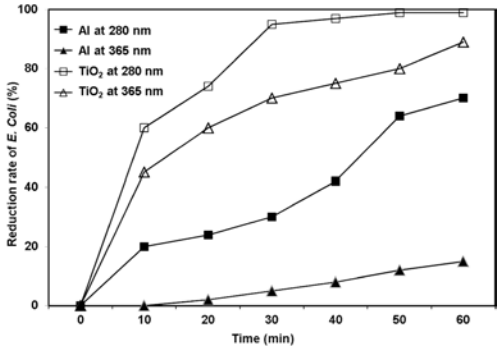

Fig. 2. Reduction rates of *E. coli* on Al substrates and TiO_2/Al as a function of wavelength.

Fig. 1(c) shows a diagram of platform for examination of antibacterial effect and photograph of the acrylic box for examination of antibacterial test. The effects of light wavelength and irradiation time on the inactivation of *E. coli* were evaluated as one of experimental parameters in assessing the antimicrobial activity. Fig. 2 shows the *E. coli* inactivation as the irradiation time varied from 0 to 60 min as a function of wavelength. From these experiments it was quite obvious that short wavelength at 280 nm increased *E. coli* inactivation. The reduction rate of *E. coli* on Al substrates at 280 nm-irradiation was 70% after being exposed for 60 min; that of the sample after irradiating at 365 nm for 60 min is only 15% of *E. coli* would be restrained.

Limited research has been conducted on the effectiveness of UV-LEDs for antibacterial test. Most of the data available are for LEDs that emit light in the

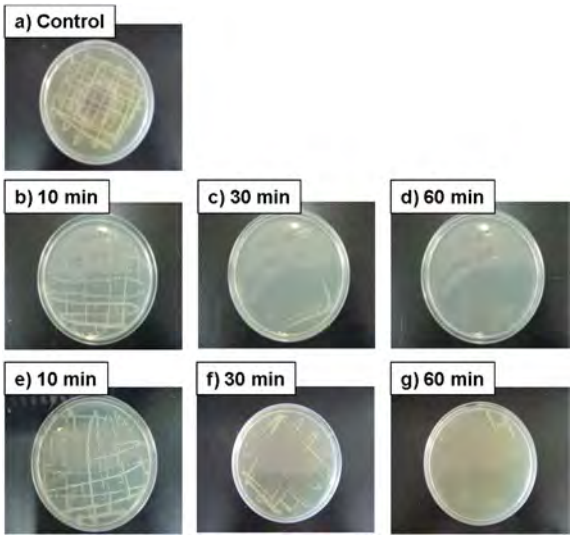

Fig. 3. The *E. coli* inactivation of the samples on TiO_2/Al as a function of wavelength [a) Control, b) at 280 nm, for 10 min, c) at 280 nm, for 30 min, d) at 280 nm, for 60 min, e) at 365 nm, for 10 min, f) at 365 nm, for 30 min, and g) at 365 nm, for 60 min].

UVA range (320 ~ 400 nm), which is less efficient at disinfection than light in the germicidal range of UVC (200 ~ 280 nm) since it is poorly absorbed by DNA [7]. UV radiation inactivates microorganisms by damaging proteins and producing hydroxyl and oxygen radicals that can destroy cell membranes and other cellular components. This process takes much more time than the damage produced by UVC, which directly affects the DNA of microorganisms by producing cyclobutane thymine dimmers, among other products, inactivating them without intermediate steps [6].

To determine the antibacterial capacity of the photocatalytic TiO_2 as a function of wavelength, we used sputtered-TiO_2 films on Al substrates. Fig. 2 shows that the reduction rate of *E. coli* on TiO_2/Al at 280 nm and 365 nm was 99% and 90%, respectively, after being exposed for 60 min. The reduction rate of *E. coli* on Al without TiO_2 coating at 280 nm for 60 min was 70%. That is to say, after irradiating for 60 min, the antibacterial performances rate of TiO_2/Al at 365 nm is higher than that of the sample without TiO_2-coating at 280 nm. Thus we proved that the photocatalytic TiO_2/Al at 365 nm have the comparable antibacterial ability to the sample irradiated by UVC. It is believed that TiO_2 thin films play a crucial role in this antibacterial activity. The antibacterial efficiency of the surface-modified substrate was obviously higher than that of the bare Al substrate.

Fig. 3 displays the results of *E. Coli* bacteria colony growth on agar plates with the different wavelength. The results clearly indicate that after irradiating at 280 nm for 60 min on the TiO_2/Al, a 99% inhibition of bacterial growth was achieved. Furthermore, as we mentioned above, for the samples irradiated at 365 nm, *E. Coli* bacterial growth on the surface-modified substrate by photo-reactive TiO_2 coatings showed a higher inhibition rate than that on bare Al substrate irradiated at 280 nm.

The semiconductor photocatalyst is excited by the appropriate electromagnetic wave to produce electron and hole pairs. The electron combines with O_2 to produce superoxide free radicals, and the hole captures the electron from H_2O in air to generate hydroxyl free radicals. Both free radicals have powerful bonding ability with bacteria and fungi. Therefore, bacteria and fungi will be suppressed with DNA damaged by superoxide and hydroxyl free radicals [8]. The oxidizing activity of hydroxyl free radicals can decompose the cell wall and the cell membrane of *E. coli* attached on TiO_2 thin films. The leakage of intracellular molecules will result in a change in the cell viability. But the percent of UV light from natural light is only 5% which results in the lower antibacterial activity. To effectively detoxify noxious organic pollutants the semiconductor photocatalyst generally requires UV light as the excitant source; the reason is that UV energy is greater than the band gap of the semiconductor, and it will deduce the electron hole pairs generated when the semiconductor is illuminated by UV.

The Sunada research group reported [9] direct evidence of cell membrane damage by the irradiation of a thin transparent TiO_2 film to examine the photocatalytic degradation of endotoxin from *E. coli*. The endotoxin is a component of the outer membrane of Gram-negative bacteria and is released only when the cellular structure is destroyed. The result indicated that the TiO_2 photocatalyst destroys the outer membrane of the *E. coli* cell and causes the death of the bacteria. Recently, the mechanism of cell killing of *E. coli* on TiO_2 thin films has been investigated by AFM [10]. The damage process of the cell wall and the cell membrane was observed by AFM imaging. Also, the permeability of the cell membrane was examined by K^+ leakage from *E. coli*. Results showed that the intracellular K^+ leaked out from *E. coli* very quickly after TiO_2 thin film was irradiated by UV. The researchers believe the cell death was caused by the decomposition of the cell wall first, and then subsequent decomposition of cell membrane [10]. Damage of the cell membrane directly leads to leakage of minerals, proteins, and genetic materials, causing cell death.

It is worth noting that, photoreactive TiO_2 coatings on Al substrates showed much higher antibacterial activity in contrast with Al substrates at 365 nm. If the TiO_2/Al will be joined to UVA-LEDs, an automobile evaporator system could be a viable and improved option. Mechanical strength will be very important for an automobile system that requires a high vibration and impact, where low pressure lamps may become difficult to install and maintain. The sterilization system is also compact and can clean air effectively, and has the potential for wide use as an air cleaner in automobile, house and factory.

Conclusions

TiO_2 coatings on Al substrates for an air-conditioner evaporator were successfully prepared and antibacterial activity was examined at 280 and 365 nm. After irradiating for 60 min, the reduction rate of *E. Coli* on the samples at 280 and 365 nm was highly increased by applying photoreactive TiO_2 coatings on Al from 70 and 15% to 99 and 90%, respectively. Photoreactive TiO_2/Al substrates showed much higher antibacterial activity in contrast with Al substrates at 365 nm. If the TiO_2-coated Al will be joined to UVA-LEDs, an evaporator system could be a viable and improved option.

References

1. C.C. Tseng and C.S. Li, Aer. Sci. Tech. 39 (2005) 1136-1142.
2. R.L. Riley and J.E. Kaufman, Appl. Microbiol. 23 (1972) 1113-1120.
3. B.H. Kim, Y.S. Jeon, J.H. Jeong, J.H. An, K.O. Jeon and K.S. Hwang, Current Appl. Phys. 7 (2007) 108-111.
4. L.J. Rose, R.B. Simmons, S.A. Crow and D.G. Ahearn, Curr. Microbiol. 41 (2000) 206-209.
5. R.B. Simmons, L.J. Rose, S.A. Crow and D.G. Aheam, Curr. Microbiol. 39 (1999) 141-145.
6. S-Q Sun, B. Sun, W. Zhang and D. Wang, Bull. Mater. Sci. 31 (2008) 61-66.
7. R. Sinha and D. Häder, Photochem. Photobiol. Sci. 1 (2002) 225-235.
8. L. Zang, W. Macyk, C. Lange, W.F. Maier, C. Antonius and D. Meissner, Chem. Eur. J. 6 (2003) 379-384.
9. M.D. Blake, P. Maness, Z. Huang, J E. Huang, Separation and Purification Methods, 28 (1999) 1-50.
10. Z. Lu, L. Zhou, Z. Zhang, W. Shi, Z. Xie, H. Xie, D. Pang and P. Shen, Langmuir 19 (2003) 8765-8768.