Developing a Testing Method for Antimicrobial Efficacy on TiO₂ Photocatalytic Products

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Abstract

TiO₂ photocatalyst has been known to exhibit a notable disinfecting activity against a broad spectrum of microorganisms. A lot of commercial TiO₂ photocatalyst products have been developed for antimicrobial purposes. However, a standard method has not yet been proposed for use in testing antimicrobial activity. In this study, we developed a TiO₂ photocatalytic adhesion test method with film as the standard testing method for the evaluation of antimicrobial activity. This method was devised by modifying the previous antimicrobial products test method, which has been widely used, and considering the characteristics of TiO₂ photocatalytic reaction. The apparatus for testing the antimicrobial activity was composed of a Black Light Blue (BLB) lamp as UV-A light source, a Petri dish as the cover material, and a polypropylene film as the adhesion film. The standard TiO₂ photocatalyst sample, Degussa P25 TiO₂-coated glass, could only be used once. The optimal initial concentration of the microorganism, proper light intensity, and light irradiation time were determined to be 10⁶ CFU/mL, 1.0 mW/cm², and 3 hr, respectively, for testing and evaluating antimicrobial activity on the TiO₂ surface.

Keywords: TiO₂, Photocatalyst, Standardization, Antimicrobial efficacy, Adhesion film

1. Introduction

Numerous studies have utilized and applied the strong oxidizing power of TiO₂ photocatalysts in environmental systems such as air purification, water disinfection and hazardous waste remediation. Since the photochemical sterilization of Escherichia coli (E. coli) using Pt-TiO₂ was first reported by Matsu-naga et al. (1985), TiO₂ photocatalysts have also been utilized to disinfect a broad spectrum of microorganisms. In an effort to commercialize TiO₂ photocatalysts, many different types of TiO₂-coated materials, such as paper, thin film and glass that exhibit great antimicrobial activities, have been prepared and evaluated. In addition, TiO₂ films deposited with antimicrobial metals, such as copper and silver, have been developed to obtain improved antimicrobial activity under mild conditions such as weak light intensity.

Although a wealth of TiO₂ photocatalyst coated products containing antimicrobial activity have already been developed and commercialized, there has been no standard method with good experimental rationale suggested for testing antimicrobial activity. The concept of not developing standard methods for testing the antimicrobial activity of TiO₂ photocatalyst was initially suggested by organizations such as the American Society for Testing and Method (ASTM), Japanese Industrial Standard (JIS), and even International Organization for Standardization (ISO), which caused the unnecessary confusion for not only the consumers but also business developers. Thus, the primary goal of this study is to develop a standard testing method for antimicrobial efficacy on TiO₂ photocatalytic products. To achieve this objective, the given standard method for evaluating the antimicrobial efficacy of various products was considered based on the characteristics of TiO₂ photocatalytic reaction. Black Light Blue (BLB) lamp was utilized as UV-A light source and the optimum experimental conditions for initial dosage of microorganism, light intensity and UV irradiation time were determined using a standard testing method with E. coli, a well-known indicator microorganism.

2. Materials and Methods

2.1. Experimental Apparatus

A simple scheme of the test method examined in this study was depicted in Fig. 1. The Black Light Blue (BLB) lamp (4 W, 4 W,
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2.2. Preparation of Materials and Photocatalytic Products

All solutions and reagents were prepared with distilled and deionized water (Barnstead NANO Pure, USA) and analytical-grade chemicals were used without further purification (Sigma-Aldrich Co., USA). All glassware was washed with distilled water and autoclaved at 121°C for 15 min prior to use. For testing antimicrobial activity in the standard TiO2 film samples, the commercial Degussa P25 TiO2 (P25) powder was used as a photocatalyst. The substrate glasses (50 mm × 50 mm) were coated with a P25 film following the procedures described in previous studies.17,18 One gram of P25 powder was vigorously mixed with 10 g of 50% carbowax binder (polyethylene glycol) aqueous solution. The mixed TiO₂ paste was deposited onto the substrate glass with two tracks of one layer of Scotch Magic Tape having 20 μm of thickness and dried under air for 30 min. Then the TiO₂-coated glass plates were heated at 450°C for 30 min to burn off the organic binder. The commercial Degussa P25 TiO₂ (P25) powder was used as a photocatalyst. The substrate glasses (50 mm × 50 mm) were coated with a P25 film following the procedures described in previous studies.17,18 One gram of P25 powder was vigorously mixed with 10 g of 50% carbowax binder (polyethylene glycol) aqueous solution. The mixed TiO₂ paste was deposited onto the substrate glass with two tracks of one layer of Scotch Magic Tape having 20 μm of thickness and dried under air for 30 min. Then the TiO₂-coated glass plates were heated at 450°C for 30 min to burn off the organic binder. The commercial samples obtained from the Korean Agency for Technology and Standards, were cut into squares of 50 mm × 50 mm and assessed after sterilizing the surface of the product with cotton soaked with 70% ethanol.

2.3. Culture and Analysis of Bacteria

For all experiments conducted in this study, E. coli was employed as the indicator microorganism for pathogenic bacteria. E. coli (ATCC strain 8739) was inoculated in 50 mL of Tryptic Soy Broth (Difco Co., Detroit, Mich.) medium in a 250 mL flask and grown for 18 hr at 37°C. The bacteria were harvested by centrifugation at 1000 × g for 10 min and washed twice with 50 mL of phosphate buffered saline (PBS, 150 mM, pH 7.1). The E. coli stock suspension was prepared by resuspending the final pellets in 50 mL of phosphate buffered saline solution. The initial populations of E. coli ranged from approximately 1 × 10⁶ ~ 1 × 10⁸ CFU/mL after diluting the stock suspension. The concentrations of cells was determined by the spread plate method, in which the cells are plated on nutrient agar, incubated at 37°C for 24 hr, and the number of viable colonies are counted.19,20

2.4. Experimental Procedures

The TiO₂ - coated glass plate was placed on a Petri dish, and then 0.5 mL of the E. coli suspension was poured onto it. The adhesion film was put on the suspension to facilitate the attachment of microbial cells to the TiO₂ surface and the cover was used to maintain the humidity at more than 90% (Fig. 1). The adhesion film was carefully pressed on the glass to prevent the E. coli suspension from spilling over the edge of the glass. After illuminating this system with BLB lamps for a predetermined time, 4.5 mL of phosphate buffered saline solution was poured into the Petri dish containing the TiO₂ - coated glass plate and adhesion film. The lamps were stabilized for approximately 30 min prior to sample illumination. The temperature of the reaction plate was held at 25°C throughout the experiment with a cooling fan. The microbial cell were detached from the photocatalytic glass plate by pipetting. This was followed by removing 1 mL of the sample. 0.1 mL of solution was withdrawn from the sample and was diluted to 1/1, 1/10, and 1/100. To count the number of viable cells from each diluted solution, triplicate plates were used. Selected experiments were repeated three times and the average value and statistical deviation were shown in the figures.

3. Results and Discussion

3.1. Light Transmittance Test of Covers and Films

Three types of covers (Pyrex, glass, and Petri dish) and three types of adhesion films [polyethylene (PE), polypropylene (PP), and acrylic] were considered as candidate materials in selecting the appropriate cover and adhesion film for TiO₂ photocatalytic adhesion test method. Fig. 2 shows the transmittance profile of three cover materials and adhesion films, in the range of 200 to 1100 nm. As shown in Fig. 2(a), all the cover materials transmitted light above 300 nm, but a different behavior in the profile was observed according to the materials used. The Petri dish was consistently transparent with a transmittance higher than 80% in a range of 300 nm ~ 1100 nm, while the transmittance of glass gradually decreased above 600 nm. The Pyrex...