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Copper-aided photosterilization of microbial cells on TiO_2 film under irradiation from a white light fluorescent lamp

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Abstract

The copper-aided photosterilization of *Escherichia coli* was examined on TiO_2 thin films under a lighting condition with less UV rays from a white light fluorescent lamp. It was found that the coexistence of Cu^{2+} and H_2O_2 in liquid phase exerted the synergistic effect on killing *E. coli* cells though these chemical species were individually at nontoxic levels to the cells (less than 10 mmol/m^3). At an incident light intensity of $I_0 = 28$ W/m^2, the addition of Cu^{2+} (10 mmol/m^3) to liquid phase on TiO_2 film gave the apparent deactivation rate constant of $k' = 12 \times 10^{-2}$ min^{-1}, the value of which was about 5 times as large as that on TiO_2 film in the absence of Cu^{2+}. This result suggested that in the liquid phase, Cu^{2+} was reduced to Cu^+ by receiving electron from photo-excited TiO_2, and then Cu^+ reacted with photocatalysis-derived H_2O_2 to produce $^\bullet$OH via Fenton-type reaction. The deactivation tests were also examined using a modified film preparation made from copper-incorporated TiO_2. On this film, the k' value was 23×10^{-2} min^{-1} at $I_0 = 28$ W/m^2, which was respectively about two and nine times as large as those on the original TiO_2 film with and without Cu^{2+} addition (10 mmol/m^3). The enhancement of biocidal activity on the modified TiO_2 film was considered to arise from promoted $^\bullet$OH formation by the aid of copper component anchored in TiO_2 solid phase.

Keywords: Titanium dioxide; Photocatalytic sterilization; *Escherichia coli*; Copper-aided reaction; Reactive oxygen species

1. Introduction

It has become of utmost concern that harmful microorganisms and viruses cause accidents of food poisoning, expansion of infectious diseases and material spoiling and so on. For maintaining our surroundings biologically clean, in recent years, TiO_2 photosterilization has attracted increasing attention because of its stable and strong oxidative reactions resulting in biocidal activity [1–8]. It has been well documented that photo-excitation of TiO_2 by UV irradiation (<410 nm) leads to generation of reactive oxygen species (ROS) such as hydroxyl radical ($^\bullet$OH) and superoxide anion ($^\bullet O_2^-$), according to the following reactions [1,2,9], and that these radical species are dominantly responsible for the biocidal activity of TiO_2 against microbes and viruses:

$$TiO_2 \xrightarrow{h\nu} TiO_2(e^- + h^+) \quad (1)$$

$$h^+ + H_2O \rightarrow {}^\bullet OH + H^+ \quad (2)$$

$$e^- + O_2 \rightarrow {}^\bullet O_2^- \quad (3)$$

$$^\bullet O_2^- + e^- + 2H^+ \rightarrow H_2O_2 \quad (4)$$

In a practical aspect of TiO_2 photosterilization system, it should be a problem to be solved that UV rays effective for photoexciting TiO_2 are very weak in living-based light sources such as a white light fluorescent lamp, which is an obstacle to attain sufficient biocidal activity of TiO_2 for inactivating biohazardous microorganisms and viruses. Recent researches have been focusing to developing photocatalysts expressing their activities in response to visible light rays [10–14]. To our knowledge, however, there have been few reports that realized high biocidal activities of photocatalysts for practical use under visible light illumination.

As an alternative approach, so far, combination of TiO_2 and metal components such as copper and silver has been developed to achieve enough biocidal activities under irradiation from light sources with less UV rays [15,16]. It has been reported that the enhancement of photocatalytic activity of TiO_2 took place through converting photo-generated H_2O_2 into more reactive $^\bullet$OH via copper-mediated Fenton-type reactions, as follows

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Nomenclature

$C_{Cu^{2+}}$	concentration of Cu^{2+} (mmol/m^3)
$C_{H_2O_2}$	concentration of H_2O_2 (mmol/m^3)
I_0	incident light intensity (W/m^2)
k'	apparent deactivation rate constant (min^{-1})
N_V	number of viable cells in reaction mixture at a given time (CFU/m^3)
$N_{V,0}$	number of viable cells in reaction mixture at $t = 0$ (CFU/m^3)
t	reaction time (min)

Greek symbol

ε	molar adsorption coefficient (m^2/mol)

[17,18]:

$$Cu^{2+} + e^- \rightarrow Cu^+ \quad (5)$$

$$H_2O_2 + Cu^+ \rightarrow {}^\bullet OH + OH^- + Cu^{2+} \quad (6)$$

In the present study, the deactivation of bacterial cells was examined using photocatalytic TiO_2 under a living-based lighting condition with a white light fluorescent lamp. To overcome the lowering in biocidal activity of TiO_2, copper component was introduced into the photoreaction system where the synergistic effect of copper and ROS can be expected to emerge even at a nontoxic copper level against bacterial cells. The aim of this work is to make quantitative evaluation of copper-aided TiO_2 photosterilization of bacterial cells under an actual lighting condition with less UV rays.

2. Materials and methods

2.1. Materials

A glass plate (76 × 52 mm, Matsunami Glass Ind., Japan) was employed as a substratum for coating TiO_2 film with or without copper component. TiO_2 sol solutions for coating were products from Taki Chemical Co., Ltd., Japan. TiO_2 film without copper component (denoted as TiO_2 film) was prepared by using TYNOC CZP223® (2% TiO_2 sol particles with 20–30 nm in diameter) and that with copper component (denoted as Cu/TiO_2 film) was made from TYNOC CSAM01®, which was a modified product of CZP223® with copper-incorporated TiO_2 particles (elemental ratio of Cu:Ti = 1:40 on a weight basis). The films were created on the glass plate by means of spin-coating at 2000 rpm for 2 s (Spincoater Type 1H-DX2, Mikasa Co., Ltd., Japan) while loading 1 cm^3 of the sol solution, followed by drying the plate at 100 °C for 10 min. The resultant preparation had an approximately 300 nm layer of TiO_2 film with mainly anatase crystallinity.

Copper chloride dihydrate and hydrogen peroxide were obtained from Wako Pure Chemical Industries, Japan. All the chemicals used were of reagent grade.

2.2. Microbial cells

Escherichia coli IM303, as reported in our previous paper [19], was used throughout experiments. The cells were cultivated at 37 °C for 6 h in an L-type glass tube with 10 cm^3 L-broth (pH 7.2) consisting of 10 kg polypepton (Wako Pure Chemical Industries, Japan), 5 kg yeast extract (Becton Dickinson, USA) and 5 kg NaCl in one m^3 of distilled water. After the cultured cells were harvested by centrifugation (10 min at 4 °C and 10,000 × g) and washed using saline solution (9 kg/m^3 NaCl), they were preserved at 4 °C until the use for deactivation tests.

2.3. Deactivation tests

The deactivation tests were conducted on the glass plate with or without TiO_2 coating on which a silicon rubber square frame (30 mm wide and 3 mm thick) was attached as a bank to give a hollow for loading reaction mixture. The glass plate was placed on a board in a dark chamber and irradiated by a common lamp used in living room, a tubular type of 20 W white light fluorescent lamp (Type FL20SEX-N/M-HG, NEC Lighting Ltd., Japan). Incident light intensity on the plate surface was measured using a photometer (Type DX-100, Takemura Electric Works Ltd., Japan) and adjusted at $I_0 = 3$ or 28 W/m^2 by changing the vertical distance between the glass plate and lamp. According to the supplier's instruction, light energy effective for TiO_2 excitation (wavelengths below 410 nm) was estimated to be less than 5% in whole light spectrum from the lamp.

In the deactivation tests, 2 cm^3 of a reaction mixture (pH 6) containing the cells of $N_{V,0} = 1.0 \times 10^{12}$ cells/m^3 in the saline solution was located in the follow framed by the silicon rubber bank on the test glass plate kept at around 25 °C, and the photoreaction was initiated by turning on the lamp.

Cell viability in the reaction mixture was estimated by means of colony-counting procedure. A sample (100 mm^3) was withdrawn from the reaction mixture at a given reaction time, followed by spreading on L-broth agar plates (1.5% agar) after needed dilution using the saline solution. After incubating the agar plates for 24 h at 37 °C in the dark, the developed colonies were numerated and the number of viable cells was recorded in terms of colony forming unit (CFU) per unit volume of the reaction mixture. The respective data were means of the values obtained from triplicate measurements.

2.4. Analytical methods

Formation of malondialdehyde (MDA) via oxidation of 2-deoxyribose by $^\bullet OH$ was used as an index for $^\bullet OH$ generation in reaction solution. MDA concentration was determined by reaction with thiobarbituric acid (TBA) to form a pink MDA-TBA adduct [20]. Two cubic centimeters of cell-free saline solution containing 2 mol/m^3 2-deoxyribose were put on the glass plate under the indicated irradiation condition and an aliquot of reaction solution (400 mm^3) was mixed with 30% trichloroacetic acid (200 mm^3) and 0.67% TBA (600 mm^3) aqueous solutions,