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Review

Visible light-assisted bactericidal effect of metalphthalocyanine-sensitized titanium dioxide films

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Abstract

Two dye-sensitized semiconductor titanium dioxide films containing copper(II) phthalocyaninechloride ($CuPcCl_{14–15}$) and copper(II) phthalocyaninetetrasulfonic acid (CuPcTs) TiO_2 films were prepared. Their bactericidal effects under visible light irradiation were measured. Experimental results indicate that these films inhibit the growth of *Escherichia coli* under visible light irradiation, and the bactericidal efficiency is related to the coverage of the TiO_2 films. A possible mechanism was proposed.

Keywords: *Escherichia coli*; Visible light; Bactericidal effect; Copper(II) phthalocyaninechloride; Copper(II) phthalocyaninetetrasulfonic acid

1. Introduction

Since Fujishima and Honda [1] reported the photocatalytic properties of TiO_2 in 1972, semiconductor photocatalysis has received a lot of attention [2,3]. Recently, reports concerning the bactericidal effects of TiO_2 have appeared [4–13]. For instance, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *Escherichia coli* were completely sterilized when incubated with platinum-loaded TiO_2 particles under metal halide lamp irradiation for 60–120 min [4,5]. TiO_2 thin films can also kill bacteria under UV irradiation [6]. TiO_2, WO_3, and co-catalyst modifications under irradiation with a combination of black light and fluorescent lamps enabled the photocatalytic inhibition of algae growth [9]. As for the bactericidal mechanism of TiO_2 nanoparticles, there are two contradictory explanations [14–18], but the photocatalytic killing mechanism has not been established yet.

TiO_2 photocatalysis is known to generate various active oxygen species, such as hydroxyl radicals, hydrogen peroxide, superoxide radical anions, etc. by redox reactions under UV irradiation. A wide range of organic compounds can be decomposed under photocatalytic conditions [2,19]. Although solar energy is inexhaustible, only a few percents of its energy are in the UV range. TiO_2 cannot be an effective bactericidal medium unless there is sufficient irradiation time under bright sunlight. Photosensitized degradation of organic compounds and solar cells based on dye-sensitized TiO_2 films have been developed [20–24]. The much-enhanced utilization of the visible light spectrum opens up many potential applications. The principle of photosensitization of TiO_2 is illustrated in Scheme 1, which indicates the primary electron pathways [25].

When a dye molecule absorbs visible light, it is excited to a higher energy state. The excited dye* then injects an electron to the conduction band of TiO_2. The injected electron is scavenged by the surface-adsorbed O_2 to yield $O_2^{\bullet-}$ and subsequently the OH radicals.

Self-disinfecting thin films have become particularly attractive in places such as hospitals. Metallophthalocyanines are very stable metal complexes and a number of properties contribute to their extraordinary versatility. These include their redox activity, high thermal stability, and non-toxicity. Potential applications include electrochromic and photocatalytic processes, such as solar energy conversion [26,27]. Copper(II) phthalocyanines are very common phthalocyanines and obtainable easily. Therefore, we chose copper(II) phthalocyanine dyes as sensitizers and prepared dye-sensitized, TiO_2 films from titanium dioxide (Degussa P25) and different phthalocyanine dyes in this work, and the bactericidal activities were measured. It was found that the films can kill *E. coli* DH5α bacteria under visible light irradiation ($\lambda > 420$ nm) effectively and the results also

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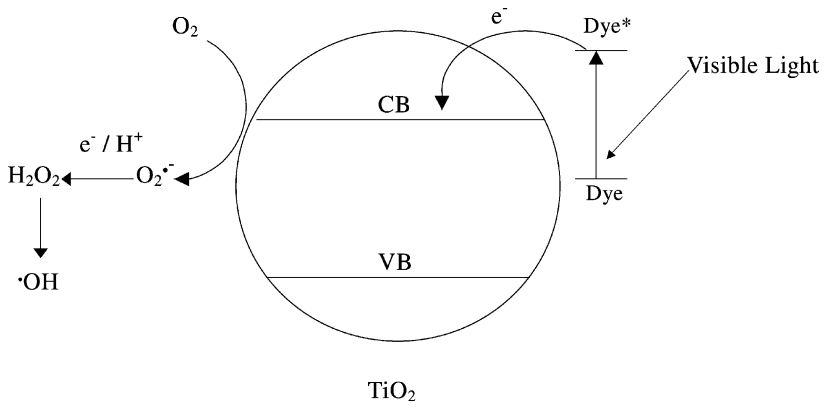


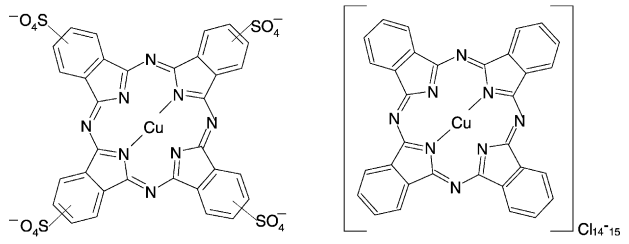
Scheme 1.

indicate that the bactericidal activity is related to the adsorption amount of the dye on titanium dioxide film. This is also a new and effective method to kill microbes in aqueous medium. An explanation for the bactericidal effect of the dye-sensitized films under visible light irradiation was proposed.

2. Experimental

2.1. Materials

Soda lime glass Petri dishes, with 60 mm diameter, were used. Titanium dioxide (P25) was from Degussa Company (Germany). Phosphorate buffer solution, copper(II) phthalocyaninetetrasulfonic acid (CuPcTs), dimethyl formamide were from Aldrich. Copper(II) phthalocyaninechloride ($CuPcCl_{14–15}$) was from Gilla Paint Company. Deionized water was used in all the experiments.



CuPcTs $CuPcCl_{14-15}$

2.2. Preparation of TiO_2 film

Commercial TiO_2 powder (P25, Degussa AG, Germany, a mixture of ca. 30% rutile and 70% anatase, BET surface area 55 $m^2\,g^{-1}$, average particle size 30 nm) (50 mg) was ground in a porcelain mortar with a small amount of water (0.5 ml) containing acetylacetone to prevent re-aggregation of the particles. After the powder had been dispersed by the high shear forces in the paste, it was diluted by slow addition of water (1.5 ml) under continuous grinding. Finally a surfactant (Triton X-100, Aldrich) was added to facilitate the spreading of the colloid on the substrate. Then the colloid was added to the Petri dish under stirring. After air-drying at room temperature, the Petri dishes were calcined for 30 min at 450 °C in air.

The *N*,*N*-dimethyl formamide solution containing $CuPcCl_{14–15}$ (3.5×10^{-5} M) and the aqueous solution of CuPcTs (2×10^{-4} M) were prepared. The prepared TiO_2 films were immersed in the dye solution. After dyeing for a certain amount of time, the film was taken out, and heated at 110 °C in the oven for 2 h. The average thickness of the films was 4.6 μm.

The optical absorption of dye adsorbed on the TiO_2 surface was recorded with a Cary 100 Scan UV-Vis spectrophotometer with a diffusive reflectance attachment.

2.3. Growth of the organism

Liquid cultures of *E. coli* (strain DH5α) were grown aerobically in Luria Broth (LB) at 37 °C on a rotary shaker (170 rpm) for 18 h. The cells were centrifuged at 6000 rpm at 4 °C for 5 min and suspended in 5 ml phosphate buffer solutions (PBS).

2.4. Light source

A 100 W tungsten halogen lamp was used. The irradiation light was filtered with a filter that cut off the light with wavelengths below 420 nm. The intensity of the illumination was 10 mW cm^{-2} on the catalyst surface during the experiment.

2.5. Irradiation and experimental procedures

The cell suspension was diluted 10^5 times, then 2 ml of the diluted cell suspension was pipetted onto the Petri dish coated with TiO_2. For each sample, 20 μl cell solution was extracted by a pipette and spread on the Petri dish containing LB culture medium, incubated for 24 h at 37 °C and then the number of colonies on the dishes were counted.

3. Results and discussion

3.1. Bactericidal activity of the dye-sensitized TiO_2 films

The metallophthalocyanine-sensitized TiO_2 films were prepared. Their absorption spectra were measured with an UV-Vis spectrophotometer. Changes in the spectra of CuPcTs adsorbed on the TiO_2 surface with corresponding changes in irradiated time are shown in Fig. 1, in which spectrum a shows the spectrum before irradiation, and spectrum b shows the spectrum after irradiation. From the above results, after 24 h of visible light irradiation both the spectra of $CuPcCl_{14–15}$ and that of CuPcTs did not change. This indicates that the films are stable enough for repeated operations. The adsorption amount of the dye on the TiO_2 surface is dependent of the adsorption time of the films in the dye solution. The adsorption amounts for $CuPcCl_{14–15}$ were found to be 1, 3, 4.5, and 12 $\mu g\,cm^{-2}$ after dyeing for 1.5, 3, 6 and 25 h, respectively.

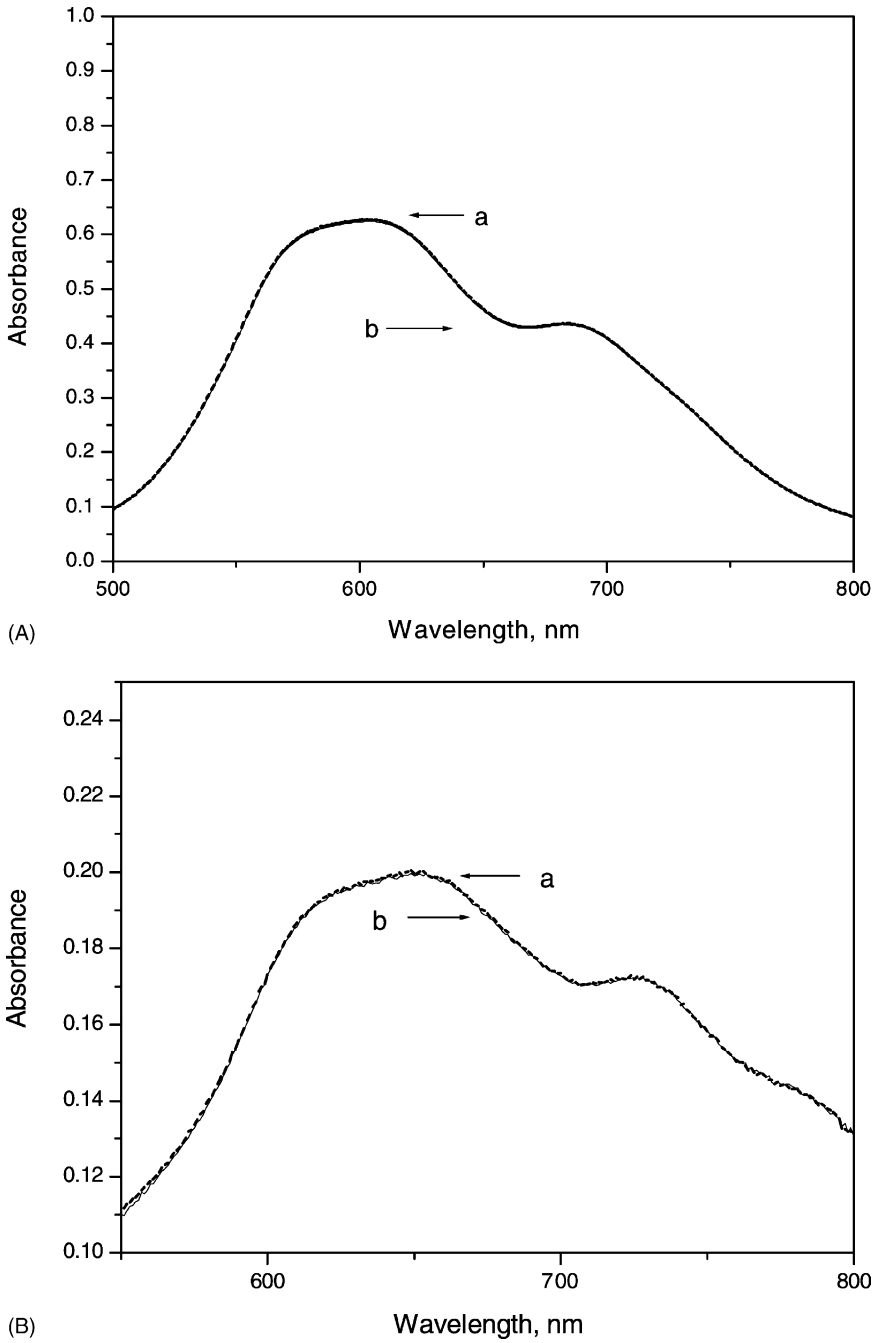


Fig. 1. Absorption spectra of CuPcTs (A) and $CuPcCl_{14–15}$ (B) adsorbed on the TiO_2 surface before (spectrum a) and after (spectrum b) visible light irradiation for 24 h.

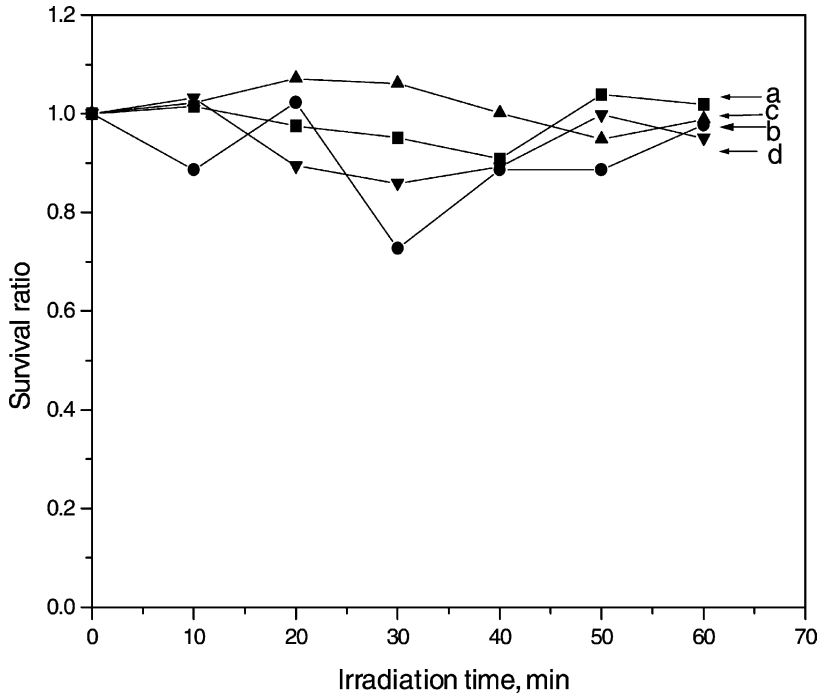


Fig. 2. Bactericidal effects of the samples: (a) TiO_2 in the dark; (b) TiO_2 under visible light irradiation; (c) CuPcTs under visible light irradiation; (d) $CuPcCl_{14–15}$ under visible light irradiation.

The photo-assisted bactericidal effects of the dye-sensitized films on *E. coli* cells were tested under different conditions. The survival ratios under the different conditions are shown in Fig. 2. The survival ratios of the bacterial cells in the system of TiO_2 put in the dark (curve a), or under visible light irradiation (curve b), or the dyes put under visible irradiation (curves c and d) did not change. These results indicate that TiO_2 alone or the dye alone has no apparent bactericidal activity under the corresponding experimental conditions. The bactericidal effect of CuPcTs in the dark was also examined. The results shown that there is no bactericidal effect on *E. coli*.

$CuPcCl_{14–15}$-sensitized TiO_2 films were tested for their bactericidal activities. Fig. 3 shows the bactericidal activities of the films prepared with different dyeing times. The results indicate that the dye-sensitized TiO_2 films have a moderate bactericidal effect on *E. coli* DH5α. About 25–70% of the bacteria were killed after 60 min of irradiation (curves a–c). The bactericidal activity decreased a little bit for the film prepared with a very long dyeing time of 25 h (curve d). This may be due to the fact that extra layers of dye molecules on the film surface inhibit the diffusion of the active species generated in the system under irradiation. The oxidative species generated on TiO_2 would be converted to stable species before they can migrate to the surface of the film. For example, some of the superoxide anion radicals donate electrons and transform to dioxygen, and the bactericidal ability would be drastically reduced. The repeated experiments also indicate the films still had bactericidal effect.

As shown in Fig. 4, the CuPcTs-sensitized TiO_2 films have higher bactericidal activities than their $CuPcCl_{14–15}$ counterparts. About 85% of the *E. coil* cells are killed in 1 h on the film prepared with a dyeing time of 6 h (curve d). The bactericidal effect, however, is dependent on the dyeing time and hence the absorbance. Similar observations have been reported in the field of dye-sensitized solar cells showing that their energy conversion were dependent on the coverage of the dye on the surface of TiO_2 films. In the aerated aqueous solution, dissolved oxygen molecules accept an electron from the conduction band of TiO_2 and are transformed into superoxide anion radicals, which react with H_2O and generate other oxidative species such as hydrogen peroxide. The concentration of the oxidative species generated directly influences the killing rate for *E. coil* cells. To explore the optimum condition to achieve photo-inactivation of bacterial cells under visible irradiation will be the objective of the future work.

3.2. Bactericidal mechanism of the films under irradiation

Since P25 is composed of rutile and anatase, the band-gap is between 3.0–3.2 eV. UV light ($\lambda < 385$ nm) may directly excite TiO_2 and produce electron–hole pairs, and also generates hydroxyl radicals and other species in aqueous solution. Under visible light irradiation (presently used, $\lambda > 420$ nm), the excited molecule in the system of metal-phthalocyanine/TiO_2 is the dye molecule but not the TiO_2 semiconductor itself:

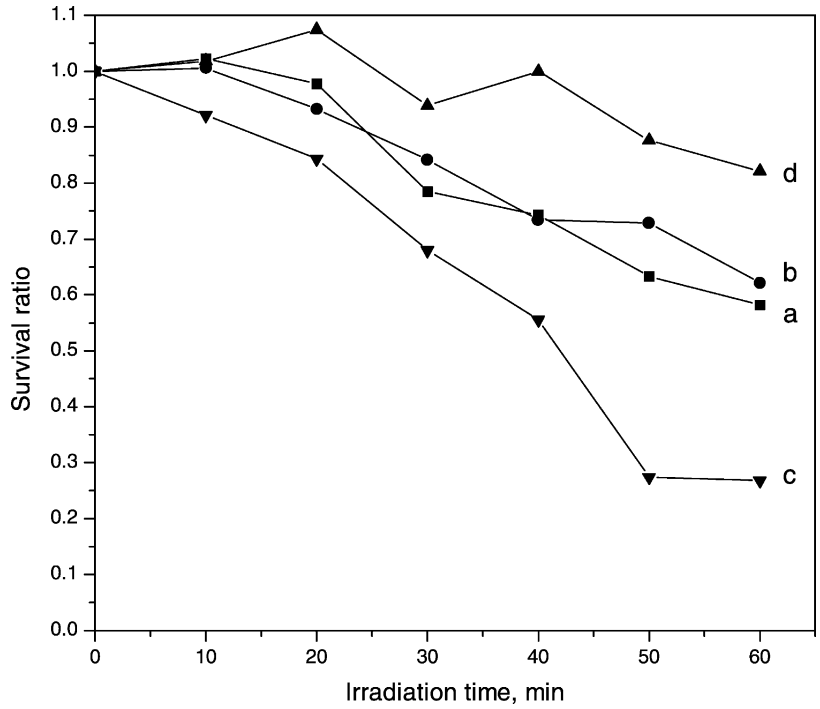


Fig. 3. Bactericidal effects of $CuPcCl_{14–15}$-sensitized TiO_2 films prepared with dyeing time of: (a) 1.5 h; (b) 3 h; (c) 6 h; (d) 25 h.

$$\text{dye} + TiO_2 \rightarrow \text{dye–}TiO_2 \quad (1)$$

$$\text{dye–}TiO_2 + O_{2,aq} \rightarrow \text{dye–}TiO_2 \cdots O_{2,aq} \quad (2)$$

$$\text{dye–}TiO_2 + h\nu \rightarrow \text{dye}^*\text{–}TiO_2 \quad (\lambda > 420\,\text{nm}) \quad (3)$$

Copper(II) phthalocyanines on the sensitized films have strong absorption around 600–650 nm, it has strong *Q*-band absorption, it can be excited on the sensitized films are excited to its single state or triple state by visible light (600–650 nm). The excited CuPcTs or $CuPcCl_{14–15}$ adsorbed on TiO_2 injects electrons to the conduction band (CB) of TiO_2. While the conduction band acts as a mediator for transferring electrons from the dye to substrate electron acceptors on the TiO_2 surface, the valence band (VB)

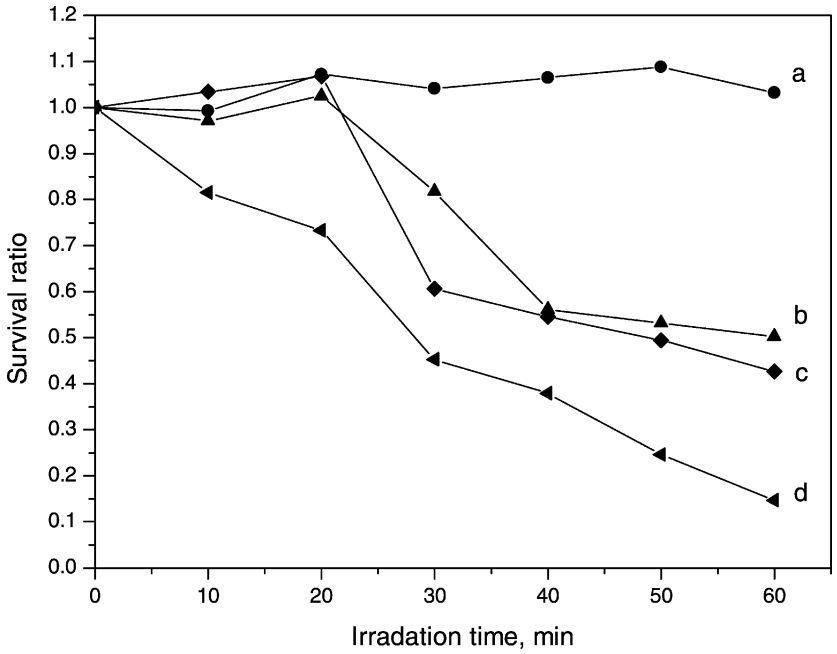


Fig. 4. Bactericidal effects of CuPcTs-sensitized TiO_2 films prepared with dyeing time of: (a) 1 h; (b) 2 h; (c) 4 h; (d) 6 h.

remains unaffected. In the presence of TiO_2, the electron injection from the excited dye to the CB of TiO_2 is faster than the direct relaxation to the ground state.

In aerated circumstances, oxygen dissolved in the aqueous solution traps an electron in the CB of TiO_2 and forms superoxide anion radicals (Eq. (4)). The generated superoxide anion radicals may react as a donor and transform into dioxygen (Eq. (5)) [28,29]:

$$\text{dye}^*\text{–TiO}_2 \cdots \text{O}_{2,\text{aq}} \rightarrow \text{dye}^+\text{–TiO}_2 + \text{O}_2^{\bullet -} \quad (4)$$

$$\text{dye}^*\text{–TiO}_2 + \text{O}_2^{\bullet -} \rightarrow \text{dye–TiO}_2 + \text{O}_2 \quad (5)$$

The generated superoxide anion radicals ($O_2^{\bullet -}$) react with H^+ in the system, which forms hydroxyl radicals, hydrogen peroxide radicals, etc. (Eqs. (11)–(14)). Hydroxyl radicals are the strongest oxidants among the generated species above that can inhibit the bactericidal effect of the system. The initial elementary reactions are listed as follows:

$$\text{dye}^*\text{–TiO}_2 \rightarrow \text{dye}^+\text{–TiO}_2(\text{e}_{\text{CB}}^-) \quad (6)$$

$$\text{dye}^+\text{–TiO}_2(\text{e}_{\text{CB}}^-) \rightarrow \text{dye–TiO}_2 \quad (7)$$

$$\text{dye}^+\text{–TiO}_2(\text{e}_{\text{CB}}^-) \cdots \text{O}_{2,\text{aq}} \rightarrow \text{dye}^+\text{–TiO}_2 + \text{O}_2^{\bullet} \quad (8)$$

$$\text{dye} \rightarrow \text{dye}^* \quad (\lambda > 420\,\text{nm}) \quad (9)$$

$$\text{dye}^* + \text{O}_2 \rightarrow \text{dye}^+ + \text{O}_2^{\bullet -} \quad (10)$$

$$\text{O}_2^{\bullet -} + \text{H}^+ \rightarrow \text{HO}_2^{\bullet} \quad (11)$$

$$2\text{O}_2^{\bullet -} + 2\text{H}^+ \rightarrow 2\text{HO}^{\bullet} + \text{O}_2 \quad (12)$$

$$\text{HO}_2^{\bullet} + \text{H}^+ + \text{e}_{\text{CB}}^- \rightarrow \text{H}_2\text{O}_2 \quad (13)$$

$$\text{H}_2\text{O}_2 + \text{e}_{\text{CB}}^- \rightarrow \text{HO}^{\bullet} + \text{OH}^- \quad (14)$$

$$\text{HO}^{\bullet} + \text{cell} \rightarrow \text{damaged cell} \quad (15)$$

The sensitized photocatalytic surface first contacts the *E. coli* cell. The generated oxidative species attack the membrane of the cells, and causing damage to the membrane and killing the bacteria. This is the key step leading to the death of the cell body. The extent of killing was inversely proportional to the thickness and the complexity of the cell wall. Some scientists proposed the detailed bactericidal mechanism of TiO_2. For instance, Saito et al. [30] proposed that the TiO_2 photochemical reaction caused disruption of the cell membrane and the cell wall. Sunada et al. [8], who studied *E. coli*, found that the endotoxin, an integral component of the outer membrane, was destroyed under photocatalytic conditions when TiO_2 was used. More direct evidence that outer membrane damage occurs was recently discovered. Maness et al. [13] reported that TiO_2 photocatalytic reaction caused lipid peroxidation to take place and as a result, normal functions associated with an intact membrane, such as respiratory activity, are lost. The oxidative damage occurs on the underlying bacterial cell after destruction of the cell membrane, photocatalytic action progressively increases the cell wall permeability. However, the damaged processes of the bacterial cells in TiO_2 photocatalytic systems are not entirely clear, and some of the explanations are contradictory. Since the photosensitization inhibition of bacteria on the surface of the copper phthalocyanine-sensitized TiO_2 films in this work probably originates from the generation of oxidative species, the destruction of the membrane of the cell is possibly the main reason for the killing of the bacteria.

4. Conclusion

CuPcTs- or $CuPcCl_{14–15}$-sensitized TiO_2 films were prepared, and the bactericidal effects under visible light irradiation were measured. According to the results of the experiments, it was concluded that the films prepared had apparent bactericidal effects on *E. coli*, and that their bactericidal activities were related to the coverage of the dye on TiO_2 surface.

Acknowledgements

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