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Enhanced inactivation of *Escherichia coli* with Ag-coated TiO_2 thin film under UV-C irradiation

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ABSTRACT

The inactivation of *Escherichia coli* (*E. coli*) in water was investigated systematically with Ag-coated TiO_2 thin film under UV-C irradiation. Compared with UV-C irradiation alone, the inactivation of *E. coli* by the UV/Ag-TiO_2 process was enhanced and the photoreactivation of the bacteria was much repressed. Moreover, atomic force microscopy (AFM) measurements of *E. coli* showed that the presence of Ag-TiO_2 thin film during UV exposure could expedite the destruction of cell wall and cell membrane, which was further confirmed by the formation of malondialdehyde (MDA) and leakage of intracellular potassium ion (K^+) and protein. The results suggest that the cell structure destruction might be the major reason for the enhancement of inactivation efficiency, and the prepared Ag-TiO_2 thin films show potential as a new improvement tool for UV-C disinfection.

1. Introduction

Water disinfection is vital for preventing the spread of diseases caused by waterborne pathogens [1]. Ultraviolet irradiation in the UV-C spectral region (190–280 nm) has been considered an emerging efficient disinfection technology, for its effectiveness in the inactivation of a broad range of pathogens and no formation of toxic byproducts in low UV dose [2].

The principal effect of UV-C irradiation in microorganisms is to damage the DNA, and the major lesion is the formation of pyrimidine dimers [3,4]. These lesions would prohibit the replication of DNA and thus resulted in inactivation of microorganisms [5]. However, some species of microorganisms have shown high resistance to UV-C irradiation [6], and many bacteria can repair their damaged DNA by light-dependent (photoreactivation) as well as light-independent (dark repair) mechanisms [7]. Therefore, the reactivation of bacteria after treatment represents a potential disadvantage for UV-C irradiation method in water disinfection.

Recently, TiO_2 photocatalysis has been proposed as one of the best disinfection technologies, for no dangerous or malodorous halogenated compounds formation [8,9]. Furthermore, evidences have shown that the photoreactivation and dark repair of bacteria can be repressed by TiO_2 modified UV-C disinfection [10]. However, traditional methods using colloidal and particulate TiO_2 catalyst suspensions are not suitable for practical water treatment, for difficulty of separation and reuse, and disinfection efficiency reduced by light screening by TiO_2 particulate in solution [11]. Thus, TiO_2 thin film, a relative new catalyst, has gained much attention [12,13].

Although the antimicrobial activity of the TiO_2 photocatalyst has been now well recognized, the mechanism leading to the photocatalytic killing of bacteria remains unclear. There are some opinions on bactericidal mechanisms of these photocatalysts, including direct oxidation of coenzymes in cells [14,15], destruction of cell structure and the damage of cell DNA [16–18]. All of these studies focused on the mechanism of TiO_2 lethality under visible or long-wave length UV light, with slow reaction rate and little effect of illumination on bacteria. The bactericidal action and the mechanism of TiO_2 thin film under UV-C irradiation have not been well investigated.

In this study, we have prepared Ag-TiO_2 thin film, a novel material, and propose a new way to take advantages of both photocatalytic oxidation and UV-C irradiation processes. The inactivation of the pathogenic bacteria *E. coli* under UV-C light irradiation with the presence or absence of Ag-TiO_2 thin film was systematically studied from the kinetic and mechanistic viewpoints. The decomposition of cell wall and cell membrane was directly characterized by AFM in conjugation with some other convincing evidence.

2. Materials and methods

2.1. Preparation and characterization of the catalyst

Microporous TiO_2 thin film was prepared directly on the surface of pure titanium (99.5 wt.%) by anodic oxidation in 1.0 M sulfuric acid. Titanium and cupper plates were used as the anode and cathode, respectively. An anodic constant current of 150 mA/cm^2

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was firstly applied on titanium surface, and then the anodic potential was increased. After the anodizing potential reached 140 V, anodizing process was carried out at a constant potential until the current decayed to stability, and the reaction was completed. The prepared TiO_2/Ti catalyst (40 mm wide, 110 mm length) was then put into 250 mL of 3 g/L silver nitrate solution (volume ratio of ethanol/deionized water equaled to 1:4), and irradiated using UV light (at 254 nm, 3.5 mW/cm^2) for 30 min with continuous nitrogen purging. The Ag-TiO_2 thin film was then washed and dried.

The crystalline structure of the film was characterized using X-ray diffraction (XRD) with a Cu Ka source. The morphology and pore distribution of the produced film were observed using scanning electron microscopy (SEM) (Camscan, MX2600FE). The thickness of the Ag-TiO_2 thin film was determined by an eddy current-based thickness gauge (CTG-10, Time Company, China).

2.2. Preparation of bacteria culture

The *E. coli* (strain DH5α) bacteria was pre-cultured aerobically in Luria–Bertani (LB) nutrient broth (which contained 10 g/L peptone, 5 g/L yeast extract and 5 g/L NaCl) at 37 °C for 20 h in a shaking incubator. After propagation, the bacterial cells were harvested from culture by centrifugation at 4000 rpm for 10 min and then washed three times with saline water (0.9% NaCl solution). Finally the resulting pellet was resuspended and diluted in ultrapure water to give a cell concentration of approximately 2×10^8 CFU (colony forming unit) per milliliter for testing.

2.3. Disinfection experiments

The experimental device used in this study is shown schematically in Fig. 1. Experiments were carried out in a pmma reactor (40 mm wide, 120 mm length and 60 mm height) with 250 mL of solution. The prepared catalyst thin film and a pure titanium plate without any treatment for control were immersed in the bacterial suspension, respectively. The *E. coli* solution was intensively mixed with a magnetic stirrer to allow a complete mixing. The reaction was carried out by overhead illumination of the bacterial suspension with a 21 W low pressure UV lamp (at 254 nm), and the exposed UV fluence was measured by a UV radiometer (Photoelectric Instrument Factory of Beijing Normal University, China). The average UV light intensity in the suspension was 2.5 mW/cm^2, which was calculated by accounting for the absorbance within the bacteria suspension and the light intensity at the suspension surface, referring to the equation described in the literature [6,19]. Before the reaction, the reactor was avoided light by a sheet of aluminum film, and the UV lamp was previously turned on for 30 min to ensure the light intensity stable. All materials used in the experiments were autoclaved at 121 °C for 20 min to ensure sterility.

Disinfection experiments for four candidate processes (Ag-TiO_2 thin film alone, UV alone, UV/TiO_2 and UV/Ag-TiO_2) were performed at pH 7.0 (adjusted with 1 mM phosphate buffer solution) and 20 °C. Experiments with different illuminated times were conducted, respectively. The bacterial suspension was transferred and used immediately for various assays at the end of the experiments. Concentration of hydroxyl radical ($OH^\bullet$) in testing solutions was determined using the *p*-chlorobenzoic acid (*p*CBA) method [20]. All the above experiments were performed in triplicate, and error bars are the standard deviation of the mean.

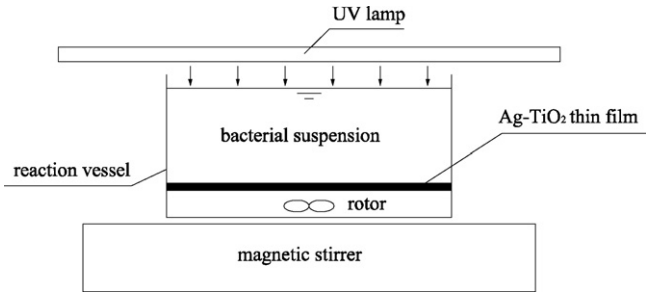

Fig. 1. Schematic diagram of equipments for testing.

2.4. Bacterial re-growth ability test

To determine the photoreactivation and dark repair of *E. coli* after treated by different processes, samples with the illumination time of 10 s were divided and transferred into two 50 mL glass flasks. One of the two flasks was exposed to white fluorescence light (1.1×10^4 lx), and the other flask was stored in the dark at 20 °C. At every time interval, 0.1 mL of the bacterial suspension was transferred and used immediately for assays. The same experiments were performed in triplicate, and error bars are the standard deviation of the mean.

2.5. Counting techniques and data presentation

The numbers of viable *E. coli* cells were counted within 30 min after sampling. A series of 10-fold dilutions were performed and 0.1 mL of each dilution was plated on LB agar plates. All plates were incubated at 37 °C for 24 h, and the numbers of colonies on the plates were counted.

Inactivation efficiency of *E. coli* η was calculated utilizing the following equation:

$$\eta = -\lg\left(\frac{N_t}{N_0}\right) \quad (1)$$

where N_0 is the initial *E. coli* population (in CFU per milliliter), N_t is the *E. coli* population remaining at time t (in CFU per milliliter).

2.6. Atomic force microscopy

E. coli cells before and after treatment were observed directly by a AFM system (BioScope, Veeco). At every time interval, 0.2 mL of the illuminated bacterial suspension was dropped on a glass slide and then air-dried for AFM imaging.

2.7. Determination of lipid peroxidation

Lipid peroxidation level was measured by the formation of malondialdehyde (MDA), which was assayed by the thiobarbituric acid (TBA) method [21,22]. Briefly, 1.0 mL of sample solution was mixed with 2.0 mL of 10% (w/v) trichloroacetic acid (TCA), and the cells and precipitated proteins were removed by centrifugation at 8000 rpm for 10 min. A volume of 3.0 mL of a freshly prepared 0.67% (w/v) TBA solution was then added to the supernatant. The samples were incubated in a boiling water bath for 10 min and cooled, and the absorbance at 532 nm was measured. The concentrations of the MDA formed were calculated based on a standard curve for the MDA (Sigma Chemical Co.), and the MDA values were given as nanomoles of MDA per milligram (dry weight (DW)) of cells.

2.8. K^+ and protein analysis

For the measurement of K^+ and protein leakage from the inactive bacteria, the illuminated bacterial suspension with different treatment times were centrifuged at 12 000 rpm and 4 °C for 10 min, the supernatant was used for assays. The K^+ was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300DV, Perkin-Elmer, Inc.). Protein was assayed by

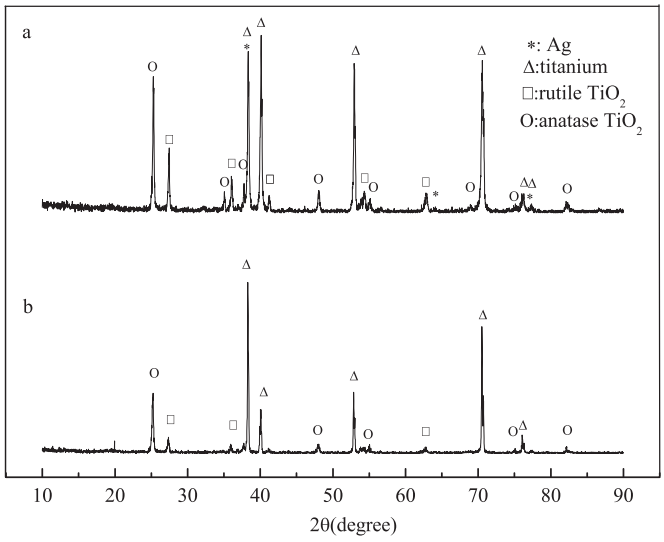

Fig. 2. XRD patterns of Ag-TiO_2 (a) and TiO_2 (b) catalysts.

the Bradford method [23], using bovine serum albumin (BSA) as standard.

3. Results and discussion

3.1. Characterization of the catalysts

The XRD patterns of TiO_2 and Ag-TiO_2 catalysts are illustrated in Fig. 2. Before Ag-loading, the pure TiO_2 thin film consisted of 79.6% anatase phase TiO_2 and 20.4% rutile phase TiO_2. The average size of TiO_2 particles obtained using Scherre formula was 35.9 nm, and the thickness of the Ag-TiO_2 thin film was approximate 2.8 μm. The 2θ values of major peaks have been found to be almost the same for Ag-TiO_2 sample when compared with pure TiO_2, and the difference was mainly in the intensities of the peaks. A peak at $2\theta = 38.4^\circ$, 64.2° and 77.4° in Ag-TiO_2 can be assigned, respectively, to (1 1 1), (2 2 0) and (3 1 1) plane of silver, which proves that TiO_2 surfaces are covered with silver particles.

The surface morphology of the two films (TiO_2, Ag-TiO_2) is shown in Fig. 3. It can be seen that the surface of films are microporous and the size of the micropore in the TiO_2 thin film ranged from 90 to 300 nm. Compared with the pure TiO_2 thin film, Ag nanoclusters are observed on the surface of the Ag-TiO_2 thin film, which is also supported by the XRD spectrum (Fig. 2).

3.2. Bactericidal activity performance study

The bactericidal activity of the Ag-TiO_2 thin film was evaluated by the inactivation of *E. coli* in water under UV light irradiation. As depicted in Fig. 4, no obvious inactivation of *E. coli* was observed in the absence of UV irradiation, which indicated that the Ag-TiO_2 catalyst is not toxic for *E. coli* in short time. For disinfection with UV alone, about 6.90 log *E. coli* was inactivated within 20 s. Some enhancement of inactivation efficiency were obtained in the UV/TiO_2 and UV/Ag-TiO_2 processes as compared to UV disinfection alone, the detected inactivation efficiencies were improved from 4.91 log to 5.44 log and 5.64 log in 10 s, respectively. These results implied that UV-C irradiation was effective in the inactivation of *E. coli*, and the use of TiO_2 thin film catalyst can enhance this effect. Although there was no obvious difference of the bactericidal activity between the TiO_2 and the Ag-TiO_2 modified UV-C disinfection process, the later resulted in more cells inactivated.

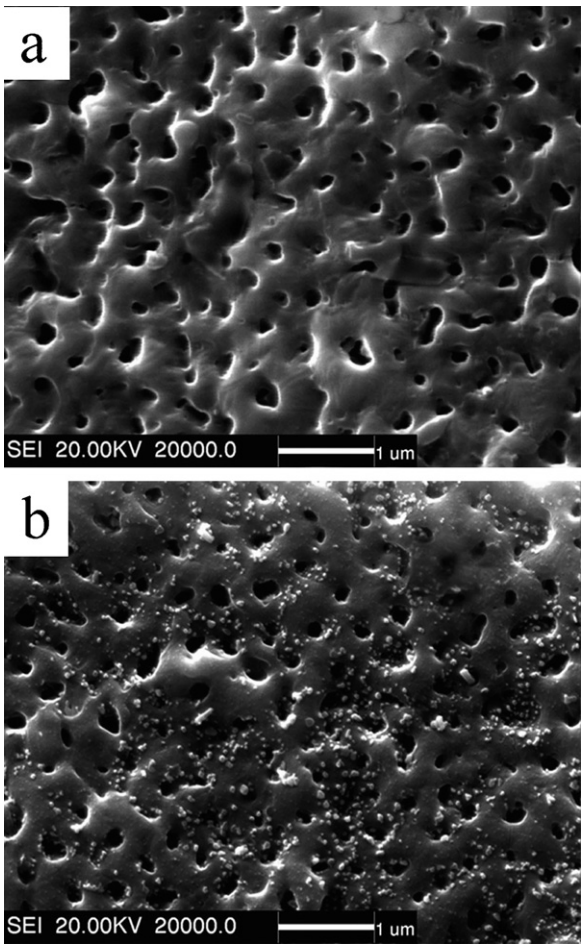

Fig. 3. SEM images of TiO_2 thin film (a) and Ag-TiO_2 thin film (b).

As suggested earlier [24,25], the mechanism of photocatalytic reactions involves the generation of intermediate OH•, and the presence of Ag atom on TiO_2 can improve the production of OH radicals. In the UV/TiO_2 and UV/Ag-TiO_2 systems, the formation of OH• was determined, and the steady-state concentrations were found to be approximate 8.4×10^{-13} and 1.1×10^{-12} M, respec-

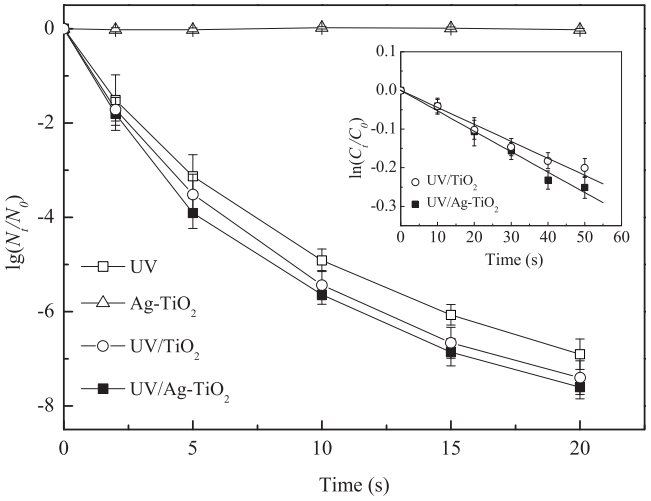

Fig. 4. Comparison of the inactivation of *E. coli* using different processes. The insert shows the time-dependent decay of *p*CBA under UV/TiO_2 (open symbols) and UV/Ag-TiO_2 (filled symbols) irradiation (UV intensity = 2.5 mW/cm^2; temperature = 20 °C).

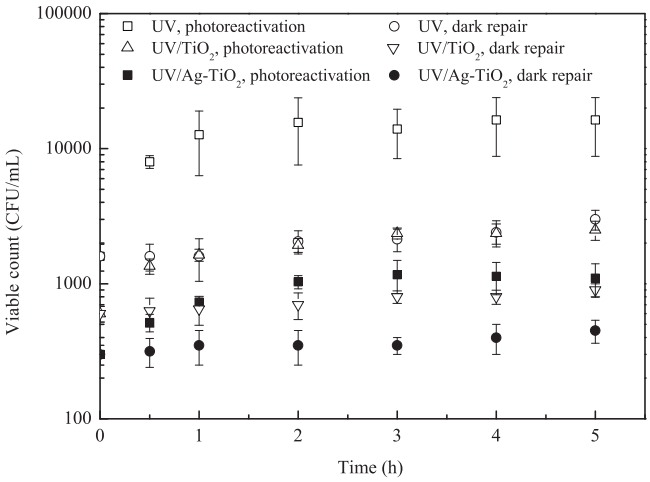

Fig. 5. Time-dependent viable counts of *E. coli* (CFU/mL) after UV irradiation alone, UV/TiO_2 and UV/Ag-TiO_2 irradiation (white fluorescence light = 1.1×10^4 lx; temperature = 20 °C).

tively. These concentrations have some coliform inactivation ability but not notable, according to the results reported by Cho et al. [20]. It should be reemphasized that the present propose is different from photocatalytic disinfection, which relies primarily on the generation of $OH^{\bullet}$ and other reactive species to give residual disinfection effect. In current study, results suggested that the UV-C irradiation plays a critical role in the inactivation mechanism of *E. coli*, and the reactive oxygen species (ROS) contribute partly to the inactivation efficiency.

3.3. Photoreactivation and dark repair of bacteria

To further evaluate the effect of Ag-TiO_2 thin film, photoreactivation and dark repair of *E. coli* after treated by UV, UV/TiO_2 and UV/Ag-TiO_2 processes were investigated. As shown in Fig. 5, photoreactivation of *E. coli* occurred in all the three samples, and the maximum bacteria counts were reached within 3 h. When treated by UV alone, after exposed to white fluorescence light for 5 h, bacteria counts were approximately 10 times higher than that obtained right after UV irradiation. In contrast, after treatment by UV/TiO_2 and UV/Ag-TiO_2 processes, photoreactivation of *E. coli* were significantly repressed, and resulted in increase in bacteria counts of less than 4 times, about 1900 and 800 CFU/mL cells reactivated, respectively, suggesting that the modification of TiO_2 with Ag nanoparticles enhanced its activity. In conditions of darkness, the reactivation occurs to a considerably lower degree than in the case of photoreactivation. After 5 h, approximate 2-fold increases in bacteria counts were obtained, compared to that at the end of UV exposure. And there was no obvious increase in bacteria counts in the presence of Ag-TiO_2 thin film during UV irradiation.

Shang et al. [10] suggested that the produced stable residual oxidants in the UV/TiO_2 system may cause some redox reactions inside the bacterial cells and subsequently repress bacteria repair. This may be a reason for the repression of photoreactivation and dark repair of bacteria after UV disinfection. On the other hand, the UV-C induced DNA damage can be self-repaired in some bacteria, while the decomposition of bacterial cell structure induced by photocatalytic was irreparable. So, the UV/Ag-TiO_2 exposure may induce additional irretrievable damage other than formation of lesions in the genomic DNA of cells. Similar hypothesis was also mentioned in previous study [10], however, has not been verified. To determine the additional irretrievable damage of bacterial cell during exposed to UV/Ag-TiO_2 irradiation, the changes of cell structure were studied, as described and discussed in the following.

3.4. Destruction of cell structure checked by AFM

AFM is a suitable method for investigation of cell morphology and structures [26]. To understand bactericidal mechanism of UV/Ag-TiO_2, the morphology of bacteria at different stages during bactericidal experiments were investigated by AFM (Fig. 6). It can be seen that in the case of no illumination (Fig. 6a and b), characteristics of the bacteria are a well-defined cell wall. Some changes had taken place to the morphology of *E. coli* that had been treated by UV/Ag-TiO_2 process for 5 s (Fig. 6c and d), there appeared some rumples and small holes in the surface of cell. The outermost layer of the cell, however, remained relatively intact. After illumination of 10 s, a large hole appeared in the cell membrane (Fig. 6e and f), and parts of the outermost layer of the cell disappeared, suggesting that cells decompose from the outside of the cell, confirmed by Sunada et al. [27]. This phenomenon was more significantly shown in the images of *E. coli* with 20 s irradiation (Fig. 6g and h). With irradiation time increasing, the quantity of holes increased and the hole size enlarged, indicating that the outer membrane of the cell has been considerably damaged, leading to an obvious leakage of the interior component.

The change of cell structure under the effect of UV alone was also presented in the figure as a reference (Fig. 6i and j). As seen in the figure, after illumination of 20 s, only a rift was observed in the outer membrane of the cell, and no disappearance of the outermost layer of the cell was detected, implying that the cell structure has not been destroyed seriously as that under the UV/Ag-TiO_2 irradiation.

The cell membrane plays a vital role in providing a barrier of selective permeability for bacteria. On the basis of the above AFM investigation, the presence of Ag-TiO_2 thin film during UV-C exposure can expedite the decomposition of the bacterial cell wall and cell membrane, indicating that the destruction of the cell structure maybe one of the important causes for the enhancement of disinfection efficiency compared to UV disinfection.

3.5. Lipid peroxidation

The level of MDA, which is interpreted as a lipid peroxidation product formed from the oxidation of cell membrane photsphatidylethanolamine, was used to estimate membrane damage under different conditions. As shown in Fig. 7, the level of MDA was comparatively low without any treatment, indicating that the amount of preexisting MDA was negligible. When *E. coli* cells were exposed to UV/Ag-TiO_2 irradiation, MDA concentration increased with the reaction time, approximately 0.42 nmol of MDA per mg of cell mass was extracted after 50 s. In contrast, irradiated by UV alone, only 0.29 nmol of MDA per mg of cell mass was detected, suggesting that the peroxidation reaction of an unsaturated lipid unit of the *E. coli* membrane was enhanced by the reactive active species generated from the system.

As reported before, both the UV-C light and the oxidants that produced during photocatalytic can induce the occurrence of an oxidative stress in cell membrane, leading to the formation of MDA [28,29]. However, with the similar disinfection efficiency, the produced MDA level in the present work was much less than the data reported in the literature that using visible or long-wave length UV light photocatalytic, and no degradation of MDA was obtained here. A possible reason, as discussed in Section 3.2, was that when treated by the photocatalytic at wavelengths of light longer than the UV-C range, the bacteria inactivation mainly depends on the attacks of ROS to cell structure, and the light nearly had no effect on the bacteria, while in the UV/Ag-TiO_2 system, the major bactericidal effect was caused by UV-C disinfection, resulting in high inactivation efficiency within shot time, and the presence of Ag-TiO_2 thin film catalyst enhanced this effect. This explanation is consistent with the results of AFM (Fig. 6), which showed that the

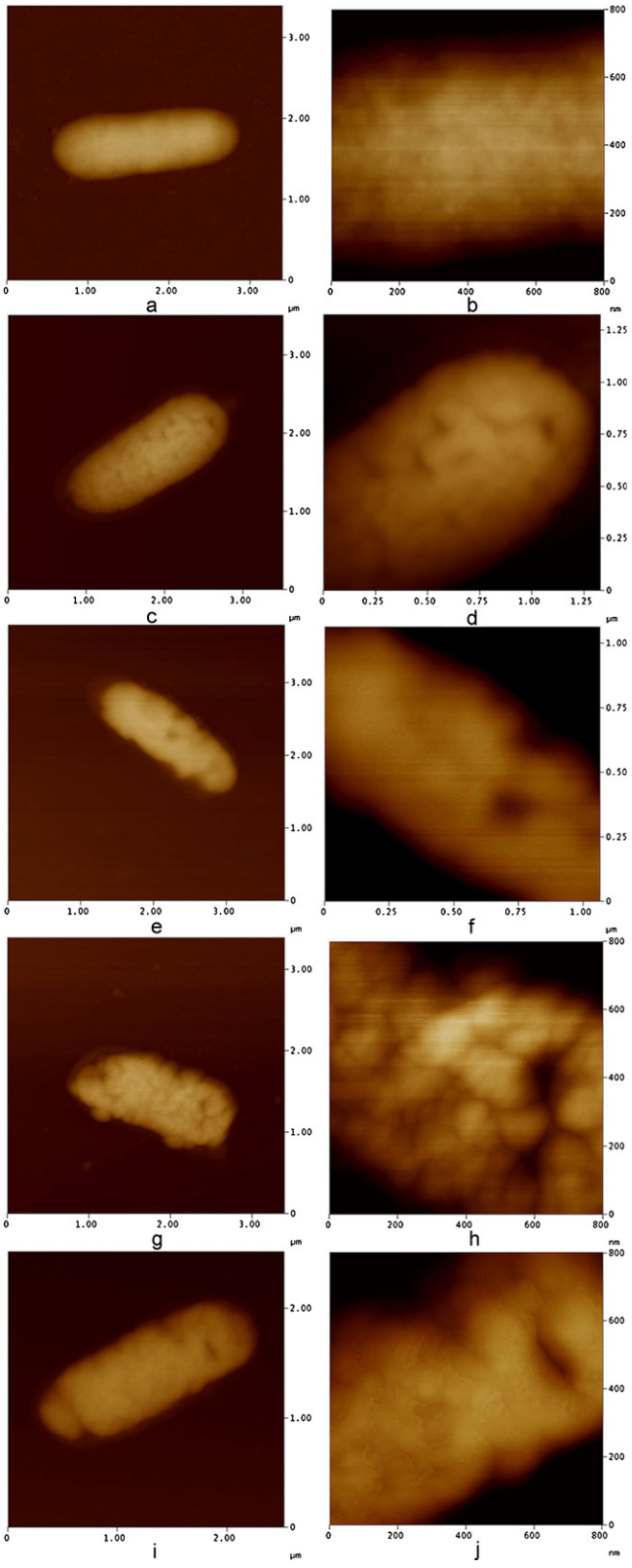

Fig. 6. AFM images of *E. coli*. Images of individual bacterium were labeled with a, c, e, g, i and their corresponding zoom-in surface structures were shown, respectively, in b, d, f, h and j. *E. coli* illuminated in the presence of Ag-TiO_2 thin film at time intervals of 0 (a), 5 (c), 10 (e) and 20 s (g); *E. coli* illuminated for 20 s in the absence of Ag-TiO_2 thin film (i).

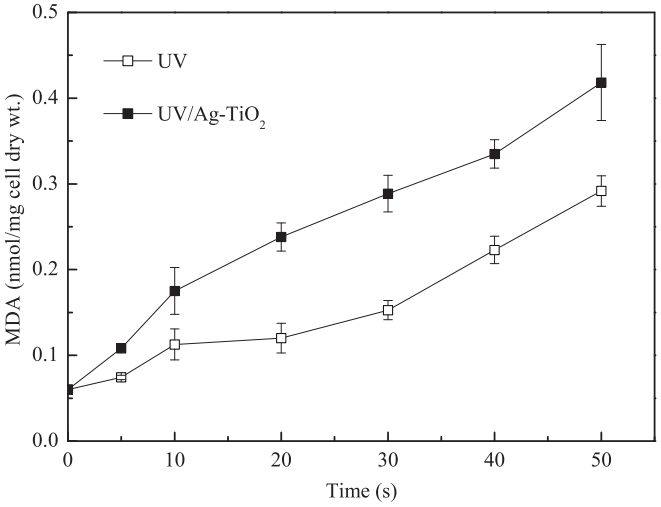

Fig. 7. Concentration of MDA from lipid peroxidation of *E. coli* changes with reaction time under UV irradiation alone (open symbols) and UV/Ag-TiO_2 irradiation (filled symbols) (UV intensity = 2.5 mW/cm^2; temperature = 20 °C).

disinfected bacterial cells have not been completely decomposed as that treated by visible light photocatalytic [17].

3.6. K^+ and protein leakage

K^+ exists universally in bacteria, and plays a role in the regulation of polysome content and protein synthesis [30,31]. In the present work, K^+ leakage from *E. coli* was used to examine the permeability of the cell membrane. Fig. 8a shows the variation of K^+ concentration with illumination time. In both the UV and the UV/Ag-TiO_2 conditions, K^+ leaked out from the bacterial cells immediately upon illumination, and the concentration increased gradually with increasing reaction time. In the presence of Ag-TiO_2 thin film, the K^+ concentration increased faster than illuminated by UV alone, approximately 111.7 and 50.6 ppb leakage of K^+ were detected after 50 s, respectively.

Lu et al. [16] suggested that with the destruction of cell structure by photocatalytic reaction, macromolecules in bacteria, such as proteins and RNAs, should also be released besides the leakage of small molecules. Thus, protein leakage from the inactivated bacteria was utilized to reflect the destruction of cell structure in this work. As can be seen from Fig. 8b, the presence of Ag-TiO_2 thin film significantly enhanced the protein leakage, and both the UV and the UV/Ag-TiO_2 processes shared similar profiles. Besides, the protein concentration increased remarkably after illuminated by UV/Ag-TiO_2 for 20 s, due to a notable change in the structure of the cell wall and cell membrane, as observed by AFM (Fig. 6g and h). After 50 s, the detected protein leakage in the conditions of UV and UV/Ag-TiO_2 were 16.9 and 24.8 ppm, respectively, indicating that the presence of Ag-TiO_2 thin film expedite the decomposition of cell structure.

According to previous research [16,17], TiO_2 photocatalytic reaction produces various reactive species ($OH^\bullet$, $O_2^{\bullet-}$), causing disruption of cell structure and the resultant leakage of intracellular components. Compared with the curves in Fig. 4, we find that the detected K^+ and protein concentrations are not proportional to account of dead bacteria. Even most of the cells had been inactivated, there was no obvious protein leakage. It should be noted that, there were three ways of the cell death in the UV/Ag-TiO_2 system. The most important way was due to the DNA damage by UV-C. The second was due to the simultaneous action of the two stimuli by UV-C and photocatlysis of Ag-TiO_2. Limited cell-killing by the photocatalysis alone was the third. The latter two ways resulted in

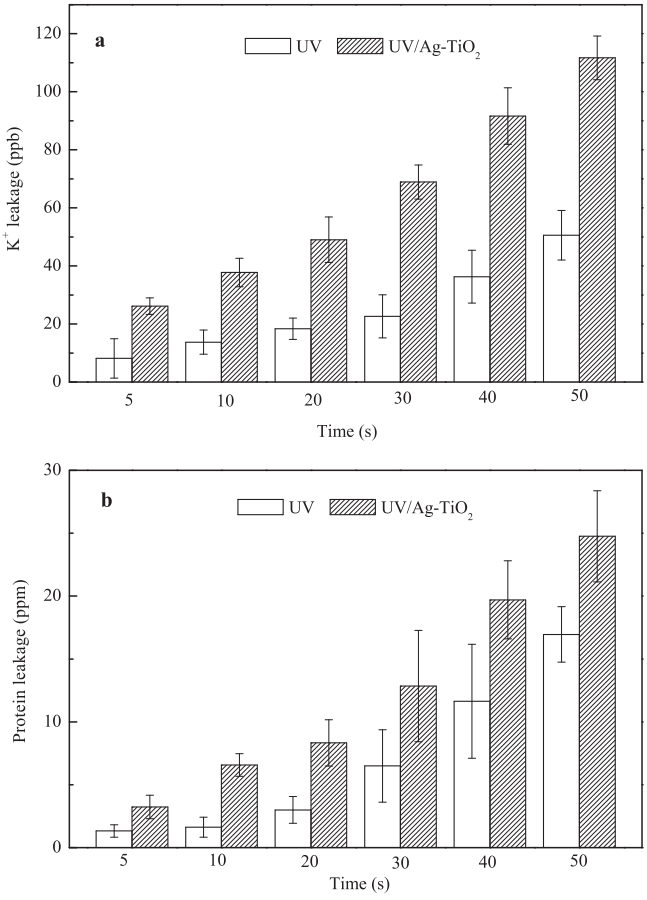

Fig. 8. Leakage of K^+ (a) and protein (b) from *E. coli* cells under UV irradiation alone and UV/Ag-TiO_2 irradiation (UV intensity = 2.5 mW/cm^2; temperature = 20 °C).

the cells disruption. Thus, the above observation can be explained by that the rate of cells disruption of *E. coli* is lower than that of the cells inactivation. This explanation agrees with the results obtained by Ren et al. [32] and Li et al. [33]. On the other hand, the K^+ and protein leakage were consistent with the decomposition of the cell wall and cell membrane, demonstrating that although the *E. coli* inactivation was mainly caused by UV-C irradiation, the ROS produced in the UV/Ag-TiO_2 system resulted in more rapid and serious the cell structure destroy, and this may be an explanation for the repression of the photoreactivation of bacteria.

4. Conclusions

A novel TiO_2 thin film was prepared directly on surface of pure titanium by anodic oxidation, followed by sliver load via photodeposition method. The UV/Ag-TiO_2 process can repress the photoreactivation of *E. coli*, with much higher bactericidal activity than UV alone. AFM observation directly evidenced gradual cell wall and cell membrane decomposition, exposed to the UV-C irradiation with the presence of Ag-TiO_2 thin film. Compared with UV irradiation alone, the MDA produced in the UV/Ag-TiO_2 system was improved, resulting in acceleration leakage of intracellular K^+ and protein. The active species, which were produced in the UV/Ag-TiO_2 system, contributed to the inactivation of *E. coli*, and enhanced the destruction of cell structure. These findings suggest that Ag-TiO_2 thin film catalyst could improve UV-C disinfection efficiency, and show a potential in water disinfection applications.

Acknowledgments

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