



SURFACE MODIFICATION OF ATO PHOTOCATALYST ON ITS BACTERICIDAL EFFECT AGAINST ESCHERICHIA COLI

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Chiang, Hshiang-Ho; Wang, Shing-Hoa; Chou, Hsin-Yiu; Huang, Chih-Ching; Tsai, Tsung-Lun; Yang, Yi-Che; Lee, Jyh-Wei; Lin, Tai-Yuan; Wu, Yeun-Jung; and Chen, Chien-Chon (2014) "SURFACE MODIFICATION OF ATO PHOTOCATALYST ON ITS BACTERICIDAL EFFECT AGAINST ESCHERICHIA COLI," *Journal of Marine Science and Technology*. Vol. 22: Iss. 2, Article 19.

DOI: 10.6119/JMST-014-0307-1

Available at: <https://jmstt.ntou.edu.tw/journal/vol22/iss2/19>

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Acknowledgements

This study was conducted with financial support provided by the Center of Excellence for the Oceans of National Taiwan Ocean University (NTOU), Keelung, Taiwan. The authors gratefully acknowledge this support. The authors also sincere thank Professor Shiu-Mei Liu at Institute of Marine Biology of NTOU for reading and correcting the manuscript with great patience

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SURFACE MODIFICATION OF ATO PHOTOCATALYST ON ITS BACTERICIDAL EFFECT AGAINST *ESCHERICHIA COLI*

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Key words: photocatalysis, ATO, nanoparticle, bactericidal effect.

ABSTRACT

The increases in catalytic photolysis efficiency of the surface modified ATO (anodic titanium oxide) by Ag or/and Au nanoparticles were attributed to the greater light absorbance than that of the sole ATO. In addition to the enhanced inherent ATO absorbance at 365 nm inasmuch as the surface-plasmon enhancement of the band emission, additional light absorbance peak occurred at the wavelength around 430 nm, 530 nm and 500 nm for nano Ag modified TiO₂, nano Au modified TiO₂, and composite nano Ag/Au modified TiO₂, respectively. All surface modified ATO catalysts exhibited stronger bactericidal effect under UV exposure than under visible light exposure for 24 hrs. Even for a short time of one hour, survival of *E. coli* decreased drastically from 106 CFU under the dark control to 102 CFU under the UV exposure, which was about four powers of exponent reduction. Surfaces modified ATO catalysts need to take longer time exposure under visible light irradiation than under UV irradiation to achieve the similar photocatalytic efficiency.

Paper submitted 10/07/13; revised 02/16/14; accepted 03/07/14. Author for correspondence: Shing-Hoa Wang (e-mail: shwang@ntou.edu.tw).

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I. INTRODUCTION

TiO₂ (Titanium dioxide) is one of the most widely studied semi-conducting photocatalysts for the degradation of organic contaminants from water and air, because of its physical and chemical stability, high catalytic activity, high oxidative power, low cost, and ease of production [10, 11, 18, 19]. Most unwanted and harmful organic compounds in contaminated air or water can be removed by photo-induced electro-chemical decomposition on the surface of TiO₂ as the photocatalyst [10]. TiO₂ photocatalysts have been found capable of killing cancer cells, bacteria, viruses, and algae under UV illumination [6, 11, 49]. Anodization of Ti substrates in a fluoride containing electrolyte leads to self-organization of the nanostructure [2, 39, 40]. These self-organized TiO₂ nanotubes exhibit very high photocatalytic efficiency that can be used to decompose organic compounds in the contact mode or in flow-through UV irradiated membranes [1, 41]. The decomposition efficiency can be further enhanced by using a bias voltage [71], or by coating the nanotubes with noble metal nanoparticles [47]. Most recently, it has been reported that living cancer cells that adhere, spread and grow on the nanotubular layers of TiO₂ surface were killed by photocatalysis. The dimensions of the nanotubes play a role in cell adhesion and spreading [48]. One possible application of the isolated nanotubes for cancer treatment is to administrate them to tumor cells, followed by focused UV-light radiation to the cells [28, 54].

The wide band gap of TiO₂ (3.2 eV) limits its use of visible light from natural solar light or room-light. Because the majority of sunlight consists of visible light, only 3-5% of them are UV light. Therefore, for the practical application of TiO₂, it is better to use visible light instead of UV light for photocatalysis. Numerous studies have been carried out to develop or improve the efficiency of TiO₂ photocatalysis by visible light radiation [3, 21, 34, 50, 60, 66], such as doping with metal and non-metal ions (e.g. Ag, Au, Pd, C, S, Pt, Fe, V, N, P) [7, 32, 35, 36, 63, 67, 70], dye sensitization (Ru, Z907, Z910, CYC-B1) [23], and semiconductor coupling. Doping

with silver is of considerable interest, since silver can trap the excited electrons from TiO_2 and leave holes for the degradation of organic species [29, 56]. Basically, doping with silver effectively narrows the band gap of TiO_2 (<3.0 eV). It also results in the extension of their wavelength response toward the visible region [4, 16, 24]. Moreover, silver particles can facilitate the electron excitation by creating a local electric field [8], and the plasmon resonance effect on metallic silver particles shows a reasonable enhancement of this electric field [23]. The effect of Ag doping through sol-gel process on titania photocatalytic activity by UV light irradiation have also been studied [69]. It has been found that Ag doping promoted the transformation of rutile to anatase, subsequently increased the specific surface area to improve photocatalytic activity and enhance the electron-hole pair separation [54]. Low concentration of metal resulted in the formation of small amount of active electron accumulating centers whereas high concentration of silver resulted in the formation of new centers of photogenerating electrons and holes, which substantially increased photocatalytic activity [38].

The processes occurring on the surface of the photocatalysts may be represented by the following explanation. Since Ag and TiO_2 have different electron energy, a Schottky barrier is formed between them in Ag/ TiO_2 catalyst. Electrons pass from TiO_2 with low electron yield energy (3.2 eV) to silver with high electron yield energy (3.0 eV). Free electron are formed on the surface of TiO_2 modified with silver particles when exposed to light, and these electrons migrate to metal particles and then to the electron acceptor (dissolved O_2) [15]. Metallic nanoparticles have attracted intensive attention due to their unusual optical properties as well as their novel chemical and catalytic properties. For example, Haruta *et al.* (1989) discovered the catalytic activity of nano-sized Au clusters on the metal oxides, such as TiO_2 , Fe_2O_3 and Co_2O_3 [15]. Among these metal oxides, TiO_2 was one of the best supports for Au nanoparticles. TiO_2 with gold nanoparticles showed high activity in photo chemical catalysis due to the improvement of photoelectron chemical activity [34]. Over the past few years, many effective approaches have been employed to prepare Au/ TiO_2 composite materials, such as by impregnation [9, 30, 37, 52], deposition-precipitation [43, 53, 68], or vacuum evaporation [42, 46]. Several kinds of active oxidative species, such as $\text{OH}\cdot$ and $\text{O}_2\cdot$ radicals, and free and trapped holes were involved in the initiation of photocatalytic oxidation reactions [27, 44, 45]. Involvement of these active oxidative species explained the superiority of TiO_2 photocatalyst to oxidize most organisms, to kill microbes, and to mineralize chemicals with the aid of molecular oxygen. These reactions mainly occurred on or very close to the surface of the TiO_2 photocatalysts. This allows important applications in the disinfection of air, water, and surfaces with TiO_2 photocatalysts. Kikuchi *et al.* (1997) studied the killing of *Escherichia coli* on TiO_2 thin films [31]. Kühn *et al.* (2003) observed that the killing efficiency on bacteria were dependent on the thickness and structure of the cell wall [33]. Bacteria

having thin cell wall, such as *E. coli* and *Pseudomonas aeruginosa*, were killed much faster than microorganisms having thick eukaryotic cell wall, such as *Candida albicans* [33]. Sunada *et al.* (1998) observed that not only bacteria were killed on the TiO_2 surface, but also the toxic ingredients could be decomposed [57]. If UV illumination continued for a sufficiently long time, bacteria were mineralized completely into CO_2 , H_2O , and other mineral substances [25, 55, 58, 62]. This is a unique property of photocatalytic sterilization not observed in other bactericidal agents. Besides bacteria, viruses and fungi also could be killed and totally mineralized by photocatalytic action [11, 58]. However, fungi were sometimes killed much more slowly than bacteria because of their cell structures [58].

Development of photocatalysts exhibiting high reactivity under visible light ($\lambda > 400$ nm) should allow the main part of the solar spectrum, or the poor illuminated interior light applicable. The purpose of this study is to develop a surface modified TiO_2 nanotube array which could kill *E. coli* under exposure of visible lights of longer wavelength. In this study, photocatalytic efficiency of nano particles of silver or/and gold modified nanotubular TiO_2 under UV and visible light were systematically investigated and compared.

II. EXPERIMENTAL PROCEDURE

1. Surface Modification of Nanotubular ATO

Titanium foil (99.7% purity, 0.127 mm thick; Aldrich, USA) was annealed in an air furnace at 450°C for 3 h to release stress and then rinsed in deionized water (DI). A platinum (Pt) sheet ($2\text{ cm} \times 2\text{ cm}$) was used as the cathode to anodize the Ti anode ($4\text{ cm} \times 4\text{ cm}$) at room temperature (25°C). Separation distance between the anode and the cathode was fixed at 1.5 cm. An ordered channel-array of nanotube layer with a length of $10\ \mu\text{m}$ was formed on the surface of titanium foil by achieving in electrolyte composed of 0.5 wt% ammonium fluoride (NH_4F , 99.9%) mixed with ethylene glycol ($\text{C}_2\text{H}_4(\text{OH})_2$) at 60 V for a period of 1 hr. After anodization, the samples were annealed at 450°C for three hrs to form a perfect channel-array of nanotubular anatase. Similar to the anodization experimental setup, the annealed foil was used as the cathode and platinum as the anode, separated by a distance of 1cm, and the foil was immersed into the electrolyte containing suspended nano silver [22] or/and nano gold [5] particles. Nano silver or/and nano gold particles were uniformly adsorbed on the titania nanotubular surface by electrophoresis under applied electric field. The electrophoretic process was performed at 30 V for nano silver particle adsorption or at 10 V for nano gold particle adsorption for 15 min. The composite nano Ag/Au adsorption process followed the aforementioned procedure for each specimen.

2. Characterization of Methodology

The structures of various samples were identified using an x-ray diffractometer (XRD) with $\text{Cu } k_\alpha$ radiation ($\lambda =$

1.54056Å). The 2θ range of XRD scan was from 20° to 80° in the measurements. The surface morphology of the samples was observed by using a scanning field-emission electron microscope (Hitachi FE-SEM 4800) with an acceleration voltage of 15 kV. The presence of nano Ag and nano Au particles was confirmed by energy dispersive X-ray analyzer (EDX). The band gap of photoluminescence (PL) for various samples was measured by using a He-Cd laser with an excitation wavelength of 340-640 nm as the light source. The Raman spectra were obtained using a solid-state green light laser (532 nm) (model Ranishaw 1000B) with an excitation power intensity of 4 mW and 15 s acquisition time. The Ultra violet-Visible (UV-VIS) absorption spectra were recorded at wavelengths of 200 nm to 800 nm on a Cary 500 Scan UV-VIS-NIR Spectrophotometer with an integrating sphere attachment using a pure titanium foil instead of polytetrafluorethylene powder as a reference. The surface topography and size of nano particle of selected samples were investigated with an atomic force microscope (AFM, DI 3100, Veeco, USA) operating in a tapping mode.

3. Preparation of Bacterial Culture

For viability assays, a typical procedure of photocatalytic treatment is used as following. *E. coli* cultivated overnight were washed and re-suspended in Phosphate Buffer Saline solution (PBS; which contains 4 g NaCl, 0.1 g KCl, 0.1 g KH_2PO_4 , and 0.571 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 1 liter, pH 7.0) to about 10^9 cfu/ml. The cell suspensions, which were irradiated by a visible-light source (0.0078 W/cm^2) or a UV-light source (0.038 W/cm^2) in the presence of the Ag on TiO_2 , Au on TiO_2 , or Ag/Au on TiO_2 photocatalyst, were pipetted onto sterile petri dishes. At regular time intervals, 20 ml aliquots of the irradiated cell suspensions were withdrawn. After appropriate dilutions with buffer, aliquots of 20 ml together with 2.5 ml top agar were spread onto agar medium plates and incubated at 37°C for 0~24 hr. The number of viable cells in terms of colony-forming units (CFU) was counted.

4. Bactericidal Tests under UV or Visible Light

Among the six 50 ml solution-samples containing 3 ml of 10^9 cfu/ml of *E. coli*, four were exposed to the TiO_2 , Ag/ TiO_2 , Au/ TiO_2 , and AgAu/ TiO_2 catalysts respectively, and the other two were served as control. One was used for the "light control" (not exposed to ATO catalyst but light only) and the other for the "dark control" (not exposed to both ATO catalyst and light). They were employed to elucidate the photocatalytic effect of various ATO catalysts. After disinfection in 95% ethanol, the various nanotube catalysts, such as TiO_2 , Ag/ TiO_2 , Au/ TiO_2 , and AgAu/ TiO_2 , were added individually into each beaker filled with *E. coli* solution. Then they were irradiated with three different light conditions, UV-A (wavelength of $\lambda = 365 \text{ nm}$), visible light, and darkness, respectively for 24 hrs after stabilization for a period of 24 hrs. The distance between the solution and the light source was fixed at approximately 10 cm. After photocatalytic treatment for 24 hrs, the solutions

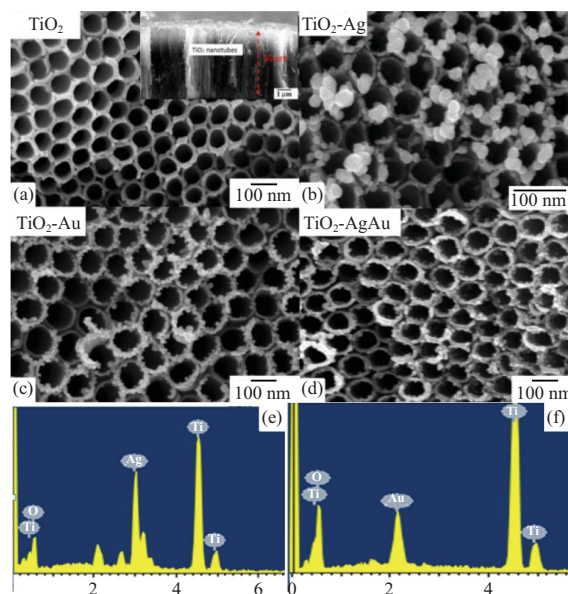


Fig. 1. SEM top views of (a) TiO_2 nanotube layer formed by anodization (b) Ag on TiO_2 (c) Au on TiO_2 (d) Ag/Au on TiO_2 (e) and (f) EDX spectra of nano silver and gold particles adsorbed on the surface of the mouth of the nanotube respectively.

containing survival bacteria were incubated for another 12 hrs. Optical density of each bacterial solution was measured by using enzyme-linked immunosorbent assay (Elisa, Synergy-Mx- Monochromator made by Biotek, USA) in order to examine the quantitative disparity between the death and viable cells. The intensity of the UV-A light was 0.038 mW/cm^2 , as measured by using a UV photometer. The intensity of the visible light was 0.0078 mW/cm^2 , as measured by using a sunlight photometer ($1\text{Sun} = 100 \text{ mW/cm}^2$).

III. RESULTS AND DISCUSSION

1. Characterization of the Surface Modified ATOs

TiO_2 with the anatase structure is more effective in photocatalysis than P25 nanoparticle oxide [12] with a major rutile structure. A modified TiO_2 -nanometer tubular array can be loaded with nano sized precious metal, and these precious metal loaded ones can be activated by the light of a longer wavelength other than the ultraviolet light. The SEM images of various nanotubular arrays in Fig. 1 revealed that the diameter of the nanotubes was 100 nm with a wall thickness of about 20 nm, and the average length of nanotubes was about 10 μm (Fig. 1(a)). That figure also showed the adsorptive silver nanoparticles (Fig. 1(b)), gold nanoparticles (Fig. 1(c)), and the composite Ag/Au nanoparticles (Fig. 1(d)) on the nanotubular wall surface. Average size of the synthesized nano silver, nano gold particles and composite nano silver/gold particles was $10 \pm 2 \text{ nm}$, $13 \text{ nm} \pm 3 \text{ nm}$, and $18 \pm 3 \text{ nm}$, respectively (Fig. 1). Composition of EDX profiles for the adsorbed nano Ag and nano Au particles on the mouth surfaces of the nanotubes were shown in Figs. 1(e) and 1(f).

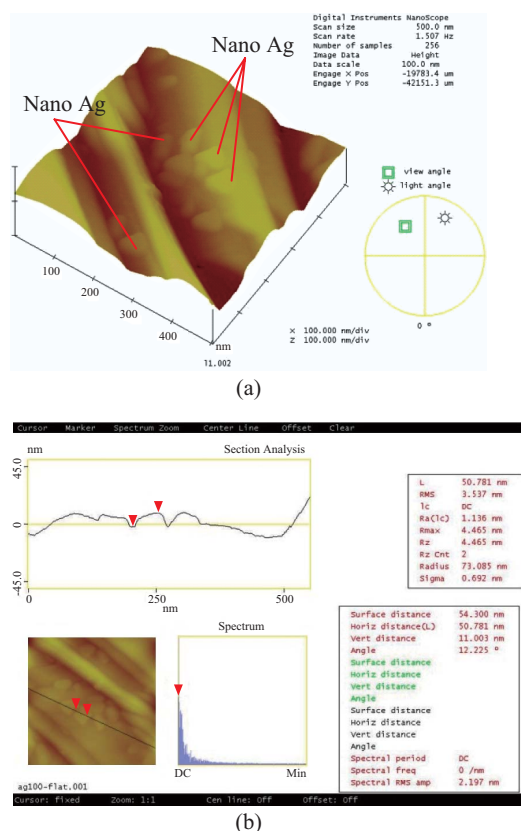


Fig. 2. AFM images of Ag/TiO₂, (a) 3D morphology, (b) size and height of nano silver particles.

Further, AFM image of a scan area (500 nm × 500 nm) taken from the surface of Ag/TiO₂ showed that nano silver particles set on a non-smooth nanotubular surface depicted in Fig. 2(a), by focusing on Ag nanoparticles. The height of Ag particle is about 13 nm measured from cross section image (Fig. 2(b)), corresponding to the aforementioned synthesized nanoparticle size. Thus this result clarifies that no anxiety about nanoparticle cluster or pile-up one on top of another occurring.

All various cases of XRD pattern plots were summarized in Fig. 3. The results showed that a nanotubular array of ATO was fully consisted of anatase (Fig. 3). Nano Ag and Au particles fully adsorbed on the mouth surface of the arrayed nanotubular wall (Fig. 1) without affecting the anatase structure were confirmed by the XRD patterns in Fig. 3. Similarly, as in Fig. 4 the Raman spectra of ATO and various catalysts, taken from the range of 100 cm⁻¹ to 1000 cm⁻¹, showed the standard peaks of anatase were at the wave numbers of 196, 395, 516, and 640 cm⁻¹, and which were consistent with the data in the literature [43]. The magnitude of the intensity raised in order of ATO, nano Ag, nano Au, and composite nano Ag/Au adsorption on ATO (Fig. 4). Surface-enhanced Raman spectroscopy (SERS) was a widely used technique for obtaining vibration spectra on some roughened metal surfaces, such as those loaded with nanoparticles. When the incident

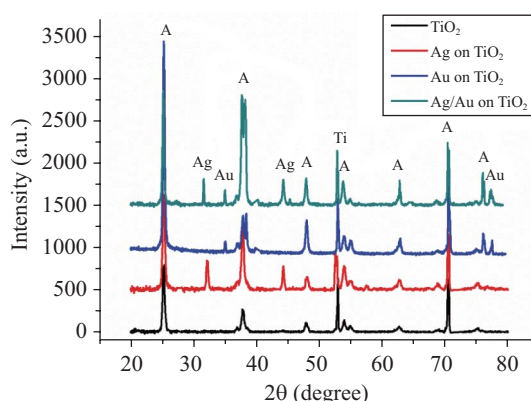


Fig. 3. XRD diffraction of ATO, Ag on TiO₂, Au on TiO₂, composite Ag/Au on TiO₂. A: anatase.

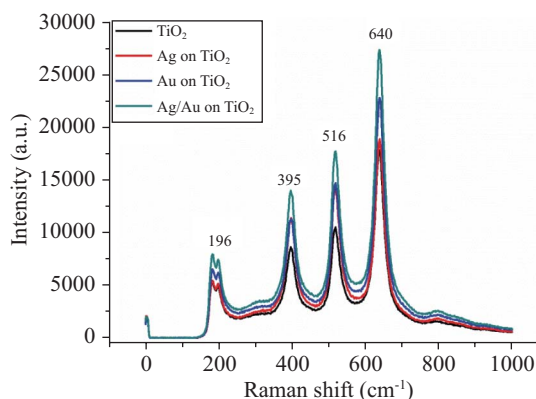


Fig. 4. Overlapping Raman spectra of the various catalysts of ATO, Ag on TiO₂, Au on TiO₂, and Ag/Au on TiO₂.

light struck and excited on the metal surface, the electric field was enhanced by the surface plasmons. The Raman signals of particles attached to the surface could thus be read to identify their chemical composition [64].

2. Light Absorbance of the Surface Modified ATOs

The light absorbance is a key factor in the efficiency of a photocatalyzer. In this study, PL spectra under low temperature 10 k were used to study the excitation wavelength and the band gap energy (Fig. 5). The anatase of ATO has an excitation wavelength of 377 nm (corresponding 3.3 eV band gap energy), which fits UV photocatalysis. The increase in intensity at 399 nm for the nano Ag adsorbed ATO might be deduced from the surface-plasmon enhancement of band gap emission by nano particles [65]. In addition to the increase in luminous intensity by surface-plasmon enhancement, band gap emission at 375 nm and one more peak appeared for absorptive nano Au particles on an ATO surface at a longer wavelength of 530 nm (2.3 eV), were consistent with the results in the literature [26]. Two maximum luminous intensity peaks occurred in the case of composite nano Ag/Au particles adsorbed on an ATO surface at 402 nm and 530 nm

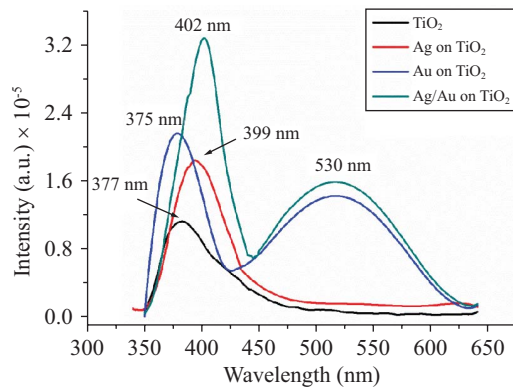


Fig. 5. The comparison of PL Spectra of the arrayed TiO_2 nanotubes with/without adsorptive different nanoparticles, excited at a low temperature of 10 K in the wavelength.

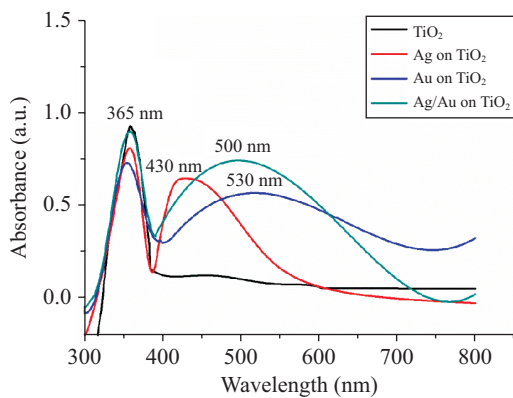
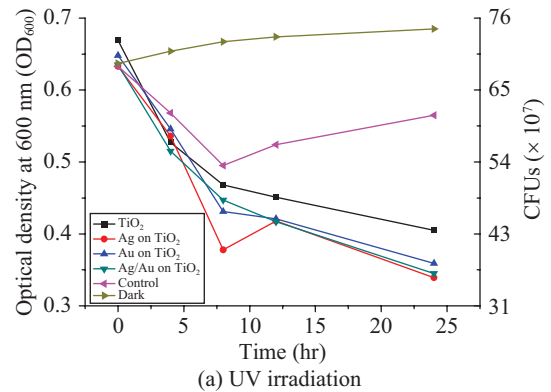


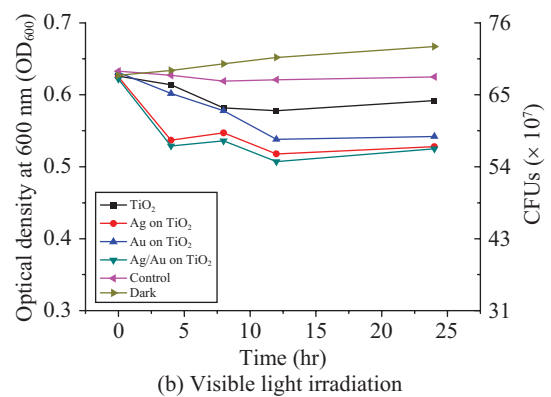
Fig. 6. The comparison of UV-VIS Spectra of the arrayed TiO_2 nanotubes with/without different adsorptive nanoparticles in the wavelength.

(i.e. 3.0 eV and 2.3 eV), with relatively lower band gap emission energy due to the highest surface-plasmon enhancement of band gap emission (Fig. 5).

Furthermore, the absorption spectra of various ATO samples were measured from UV to the visible light range with a UV-VIS spectrophotometer. The results in Fig. 6 illustrated that the maximum absorbance peak occurred at a wavelength from 355 nm to 365 nm were originated from the ATO. Additional peak at 430 nm was from the absorptive Ag nanoparticles on the ATO surface, and the peak at the longer wavelength of 530 nm was from the adsorptive Au nanoparticles on the ATO surface (Fig. 6). The light absorbance value of ATO with the adsorptive Ag nanoparticles was better than that with the adsorptive Au nanoparticles (Fig. 6). The composite nano Ag/Au particles adsorbed on ATO surface exhibited the highest of light absorbance about 0.75 at the wavelength of 500 nm, which was in the middle of a broad wavelength range covering from 430 nm to 530 nm. It was the consequence of the superimposing effect of two absorption peaks from Ag and Au nanoparticles, in addition to the inherent ATO absorbance at 365 nm (Fig. 6).



(a) UV irradiation



(b) Visible light irradiation

Fig. 7. Photocatalysis of various surfaces modified ATO on the *E. coli* bactericidal action under (a) UV-A light (b) visible light exposure.

3. Bactericidal Effect of the Surface Modified ATOs

Plots of the optical density (OD_{600}) versus the irradiation time were used to evaluate the bactericidal efficiency of photocatalysis in terms of the survival of *E. coli*, by various modified ATO catalysts under the different treatments (Fig. 7). The results showed that the bactericidal efficiency by UV-A exposure was strongly enhanced by the adsorptive nano Ag or/and nano Au particles (Fig. 7). The bactericidal efficiency was excellent under UV-A light irradiation for all surface modified ATO photocatalysts by Ag, Au, or composite Ag/Au nanoparticles. Bactericidal effect by visible light irradiation was also enhanced to contain level for all surface modified ATO catalysts (Fig. 7). Although bactericidal effect on *E. coli* cells under visible light exposure is not as effective as under UV-A exposure (Fig. 7) for each ATO photocatalysts. The photocatalyst of composite Ag/Au nanoparticles adsorbed on the ATO surface for visible light irradiation had the best bactericidal result after 24 hrs of irradiation. The increases in efficiency of the bactericidal effect in vitro was, in rising order, dark control, light control, TiO_2 (ATO), Au on TiO_2 , Ag on TiO_2 and composite Ag/Au on TiO_2 .

Bactericidal activities based on optical density (OD) measurement were semi-quantitative evaluation (Fig. 7). Manual counting of the survival *E. coli* (Fig. 8) has completed a more accurate result of photocatalytic bactericidal effect under ultraviolet and visible light irradiation for 1 hr. Each experiment

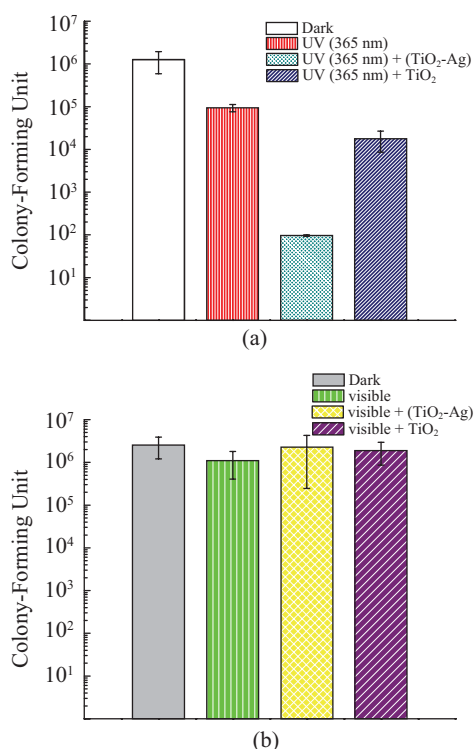


Fig. 8. Effect of (a) ultraviolet light and (b) commercial 13-Watts visible light irradiation for one hour on bactericidal effect against *Escherichia coli* by photocatalytic activities of different modified ATO surface.

had been done in triplicate (Fig. 8). The most effective photocatalytic bactericide was the surface modified ATO with nano Ag adsorption (Fig. 8(a)). The results showed bacterial colonies decreased drastically from 10⁶ CFU under the dark control to 10² CFU under the UV exposure, which are about four powers of exponent reduction in the survival *E. coli* within only 1 hr (Fig. 8(a)). For comparison, any of the selected photocatalysts was unable to demonstrate a rapid bactericidal effect under the visible light irradiation for 1 hr (Fig. 8(b)). In other words, these surfaces modified ATO photocatalysts have to take longer time under visible light irradiation than under UV irradiation to achieve a significant photocatalytic activity in current stage. In the future, the improvement of bactericidal effect under visible light irradiation is another important key issue.

The increase in photocatalytic efficiency of the nano Ag or/and nano Au absorptive ATO photocatalysts suggested that the stronger photolysis was result from the high electron conductivity of nano Ag or/and nano Au, combined with the quantum effects of the nano-sized TiO₂ tubes. The increase of the PL (Fig. 5) emission peak intensity and the UV-VIS (Fig. 6) absorption peak intensity were caused by the nano Ag or/and nano Au adsorption due to the surface-plasmon enhancement of ATO band gap emission. The so-called redshift of the additional second peak was result from the nanoparticles induced band emission having a narrow band gap, thus with the con-

sequence of having more photoelectrons from ATO (TiO₂) conveyed to the buffer solution to enhance the multiplication in the number of hydroxyl radicals and super oxides [61]. From the literatures [13, 14, 17, 20, 51, 59], *E. coli* cells treated with photo excited ATO were effectively killed due to the accumulation of reactive oxygen species (ROS) on the surfaces of cell membranes and in the cytoplasm. According to these characteristics, it had been assumed that the mechanism of photo excited ATO in bactericidal photocatalysis might be a series of oxidized chain reactions, inducing cell death by reactive oxygen species.

IV. CONCLUSIONS

The additional peak of light absorption at wavelength of 430 nm and 530 nm for surface modified ATO by Ag and Au nanoparticles might be deduced from the surface-plasmon enhancement of band gap emission by these nanoparticles. The composite nano Ag/Au particles adsorbed on ATO surface exhibited the highest of light absorbance (about 0.75) at the wavelength of 500 nm, it also had a broad wavelength range covering a range from 430 nm to 530 nm. Its bactericidal activity was the consequence of the superimposing effect of two absorption peaks from foregoing mentioned Ag and Au nanoparticles and the enhanced inherent ATO absorbance at 365 nm.

The bactericidal efficiency tests (based on OD) showed that photocatalysis of these surface modified ATO photocatalysts after UV irradiation for 24 hrs were strongly enhanced. For comparison, the bactericidal efficiency of these ATOs under visible light irradiation only increased to certain level. Bactericidal effect of these ATOs under visible light irradiation was in the increasing order of TiO₂, nano Au adsorptive TiO₂, nano Ag adsorptive TiO₂, and composite nano Ag/Au adsorptive TiO₂.

A more accurate bactericidal results by manual counting the number of the survival *E. coli* showed CFU/ml decreased drastically from 10⁶ under the dark control to 10² under the UV exposure, which was about four powers of exponent reduction in the survival *E. coli* within only one hour for nano Ag modified ATO catalyts. For comparison, it was unable to demonstrate a rapid bactericidal effect under the visible light irradiation at the same short time period. In other words, these surfaces modified ATO catalyts of this study had to take longer time under visible light irradiation than UV irradiation to achieve a significant photocatalytic activity in current stage.

The increases in catalyzed photolysis efficiency of the surface modified ATO by Ag or/and Au nanoparticles were attributed to the enhancement of much greater light absorbance than the sole ATO.

ACKNOWLEDGMENTS

This study was conducted with financial support provided by the Center of Excellence for the Oceans of National Taiwan

Ocean University (NTOU), Keelung, Taiwan. The authors gratefully acknowledge this support. The authors also sincerely thank Professor Shiu-Mei Liu at Institute of Marine Biology of NTOU for reading and correcting the manuscript with great patience.

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