Visible Light Antibacterial Activity of TiO_2-Ag Prepared from Radiophotography Wastewater

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ABSTRACT: *This paper deals with the study on the antibacterial activity of TiO_2-doped Ag prepared from radiophotography wastewater. The antibacterial agent was prepared by reduction of Ag(I) in the radiophotography wastewater over TiO_2 photocatalyst under UV light irradiation and characterized by EDS, XRD, SRUV, and TEM machines. The antibacterial activity in inhibiting the growth of Staphylococcus aureus was examined by counting the number of viable bacterial colonies using the TPC method. The result shows that Ag doping on TiO_2 as TiO_2-Ag can shift its absorption into the visible region. TiO_2-Ag assigns better antibacterial activity compared to TiO_2 under visible light irradiation. The efficiency of the antibacterial activity is found to be influenced by Ag loaded in the TiO_2, irradiation time, and the antibacterial agent dose. The highest antibacterial activity is achieved by 100 mg/L of TiO_2-Ag (2) under 3 h irradiation by visible light.*

KEYWORDS: *TiO_2-Ag; Radiophotography wastewater; Photoreduction; Silver ion; Inhibition, Staphylococcus aureus.*

INTRODUCTION

Antibacterial agent or disinfectant, a substance that can inhibit or kill the bacteria. The antibacterial materials frequently used are chlorine, ozone, and OH radical. Chlorine is very effective in killing bacteria, but it has mutagenic and carcinogenic properties (1). Ozone can combat bacteria and fungi effectively, but it is an unstable gas and requires expensive installation (2). OH radicals can be obtained from TiO_2 irradiated by UV light through a photocatalytic reaction (3). TiO_2 is a very active, low-cost, and non-toxic photocatalyst. As a photocatalyst, it has wide band gaps, i.e. 3.2 eV for the anatase phase. TiO_2 requires UV light for activation, which causes the limitation of the application of TiO_2 photocatalyst as an antibacterial agent.

Doping a metal into TiO_2 as an effort to increase the activity of TiO_2 under visible light has attracted much attention. Metal dopants that have been reported to increase the photocatalytic activity of TiO_2 are Fe, Cu, Cr, Co, and Ag (4,5). Among these dopants, only Ag metal has antibacterial activity (6). Accordingly, TiO_2-Ag demonstrated high antibacterial activity under visible light due to the double action from TiO_2 and Ag. The preparation of TiO_2-Ag was carried out by chemical reduction methods (7), sol-gel (8), and photoreduction (9). Of the methods, photoreduction is the most efficient and simplest method.

In the preparation of TiO_2-Ag by various methods, $AgNO_3$ solution intensively used as an Ag precursor

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although it is costly. In order to replace the expensive Ag source, the use of radiophotography wastewater containing high concentration ion complexes $[Ag(S_2O_3)]^{2-}$ (1-10 g/L)(10) has been reported. The study found that TiO_2-Ag was able to kill *E. coly* in well water effectively. In this study, we focus on the TiO_2-Ag preparation by using radiophotography wastewater as Ag source and its performance in the inhibiting the growth of gram-positive bacteria (*Staphylococcus aureus*).

EXPERIMENTAL SECTION

Materials

Titanium dioxide (TiO_2) powder and silver nitrate ($AgNO_3$) were purchased from Merck and used without further purification. Double-distilled water was used as the solvent. The radiophotography wastewater was collected from PDHI hospital. For the antibacterial assay, *Staphylococcus aureus* InaCC B4 was used as the bacteria test, while nutrient agar and nutrient broth purchased from Merck were utilized as the media. A set of photocatalytic reaction apparatus for batch Ag(I) photoreduction was used. The characterization was conducted using Atomic Absorption Spectroscopy (AAS, Perkin Ermer type 3110), Shimadzu-IR machines (FTIR, Prestige 21), UV-Vis Spectrophotometer with additional Specular Reflectance (SRUV, Shimadzu UV-1700 Pharmaspec), X-Ray Diffraction (XRD, Shimadzu 6000), and Transmission Electron Microscopy (TEM, JEOL JEM-1400).

Methods

Preparation of TiO_2-Ag

The photocatalyst was prepared by photoreduction method, followed the procedure reported previously (9). A series of TiO_2 as much as 500 mg was suspended in 200 mL of double-distilled water and added by 50.0 mL of radiophotography wastewater. Then, the mixture was sonicated for 1 h and stirred for 30 min. The resulted suspension was placed in a UV reactor, irradiated with UV light, and stirred for 24 h. After 24 h, the white suspension was converted to the grey suspension. The grey suspension was placed in the darkroom for 24 h before being filtered with Whatman 42 paper. The filtrate was analyzed using AAS to measure the residual Ag(I) ion concentration. The same procedure was done for various initial concentrations of Ag(I) ion in the radiophotography waste as much as 31.12, 62.28, 155.7, and 778.5 mg/L. From AAS data, the Ag formed and deposited on TiO_2 can be calculated. The solids were coded as TiO_2-Ag (1), TiO_2-Ag (2), TiO_2-Ag (3) and TiO_2-Ag (4) with the amount of Ag deposited are 15.35, 21.30, 62.30, and 270.7 mg/g respectively. The solids were dried and characterized by XRD, SRUV, and TEM.

Antibacterial activity assays

For antibacterial activity assay of TiO_2 and prepared TiO_2-Ag, *Staphylococcus aureus* (*S. aureus*, InaCC B4) was selected as the model of gram-positive bacteria. All apparatus used were sterilized in autoclave before used. The bacteria with OD 0.50-0.60 at 600 nm, which corresponds to about 10^7 colony-forming unit (cfu)/mL, were suspended in 5 mL of aqueous TiO_2-Ag suspension in nutrient broth and stirred using a magnetic stirrer. At intervals of 1 h, aliquots of the suspension were withdrawn and spread-plated on nutrient agar plates after appropriate dilutions with sterile double-distilled water. The number of viable bacteria was determined by counting the number of colony-forming units and multiplying it with the dilution factor after 24 h of incubation at 37 ºC(11). Pure TiO_2 was used as control studies. The effectiveness of the growth inhibition was represented by percentage (%), that was calculated based on the relationship of:

$$Inhibition\ level = \frac{C_o - C_x}{C_o} \times 100\%$$

where,

C_o = The initial number of bacterial colonies

C_x = The number of growing bacterial colonies

RESULTS AND DISCUSSION

Characterization of TiO_2-Ag

Based on the results of the AAS analysis, the initial concentrations of Ag(I) in the radiophotography wastewater is 1557 mg/L. The wastewater was diluted into various lower concentrations, that was further photoreduced and doped on TiO_2 structure. Fig. 1 showed the amount of Ag deposited on the surface of TiO_2 with various initial concentrations of Ag(I) in the photoreduction process. It can be seen that the increasing initial concentration of Ag(I) in the radiophotography wastewater, can enhance the amount of Ag loaded on TiO_2. Increasing initial Ag(I) concentration with the abundance electrons from TiO_2 allows the effective contact between electron and Ag(I) ion that promotes fast reduction.

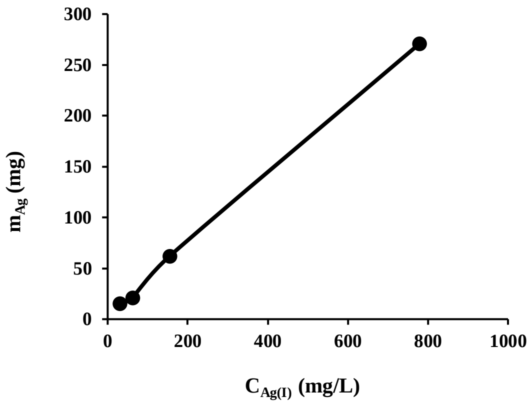

***Fig. 1:** The influence of the initial concentration of Ag(I) in the radiophotography wastewater to the amount of Ag deposited on TiO_2.*

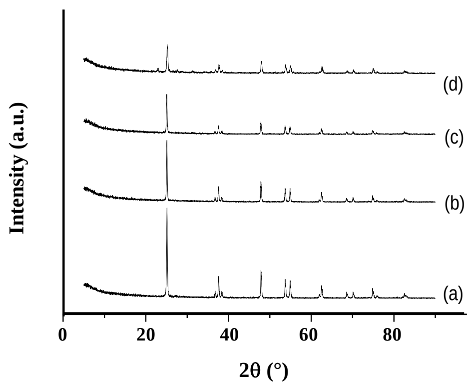

***Fig. 2:** XRD pattern of (a) TiO_2, (b) TiO_2-Ag (1), (c) TiO_2-Ag (2), and (d) TiO_2-Ag (4).*

The photoreduction of Ag(I) catalyzed by TiO_2 are presented in reactions (1) and (2).

$$TiO_2 + UV\ light à TiO_2 + h^+ + e^- \quad (1)$$

$$Ag^+ + e^- \rightarrow Ag \quad (2)$$

The XRD analysis of TiO_2 and TiO_2-Ag were presented in Fig. 2. It showed that TiO_2 probes diffraction characteristic peaks of anatase (JCPDS No. 21-1272). The XRD patterns of prepared TiO_2-Ag were similar to that of pure TiO_2, but the lower intensities are observed. Furthermore, the decrease of the intensities is proportional to increasing the amount of Ag deposited on TiO_2 (12). The Ag atoms may be inserted in the TiO_2 lattice that leads to the partially distortion of TiO_2 crystal, assigned by the decrease of intensities in XRD pattern (13). No Ag peaks were detected in Fig. 2, that can be caused small amount of Ag deposited on TiO_2 and/or in amorphous phase , that cannot be detected by XRD (14).

The TEM images and SAED of TiO_2 and prepared TiO_2-Ag (2) displayed in Fig. 3. It is observable in the TEM image that TiO_2 particles are spheres with sizes between 60 – 90 nm. TEM image of TiO_2-Ag (2) has dark irregular particles, representing Ag particles on TiO_2 with sizes between 5 – 10 nm. One study reported similar finding (15). Further, Selected Area Electron Diffraction (SAED) analysis was used to confirm the present of Ag on TiO_2 structure. Based on the crystal lattice distance (d) from JCDPS No. 21-1272 corresponding to Ag, it is attributed that d of TiO_2-Ag (2) calculated from SAED is belonged to Ag. It confirms that the dark irregular spheres on TEM image are Ag particles. The observable irregular shapes of Ag indicates that Ag on TiO_2-Ag is formed as amorphous phase that undetectable by XRD(9).

The effect of Ag doping on the light absorption of TiO_2 was analyzed by SRUV giving the absorption wavelengths and band gap energy (E_g) values, that were represented in Table 1.

From the table, it appears that doping Ag on TiO_2 can shift its absorption into longer wavelength, that falls into the visible spectrum. The shift is due to the Ag insertion into the TiO_2 structure narrowing the gap between conduction and valence bands(16). The narrowing is indicated by lower E_g values.

Antibacterial activity assay

The antibacterial activity assay of TiO_2-Ag was conducted under the irradiation of visible and dark condition for comparison purpose. The antibacterial effectiveness of TiO_2 and TiO_2-Ag prepared at different conditions are presented in Fig.5 .

The Figureure displayed that at dark condition very low antibacterial activity TiO_2-Ag is observed, and no activity is shown by TiO_2. The antibacterial is only contributed by Ag doped in TiO_2. Ag has several mechanism of antibacterial acivity, such as destabilizes and increases the permeability of bacterial membrans, inactivates sulphur containing essential respiratory enzymes, and distrups ion transport processes that kill the bacteria(11). TiO_2 is not able to combat the bacteri

Table 1: Energy band gap of TiO_2-Ag obtained

Sample	Wavelength (nm)	E_g (eV)
TiO_2	385	3.22
TiO_2-Ag (1)	392	3.16
TiO_2-Ag (2)	409	3.03
TiO_2-Ag (3)	391	3.17
TiO_2-Ag (4)	395	3.14

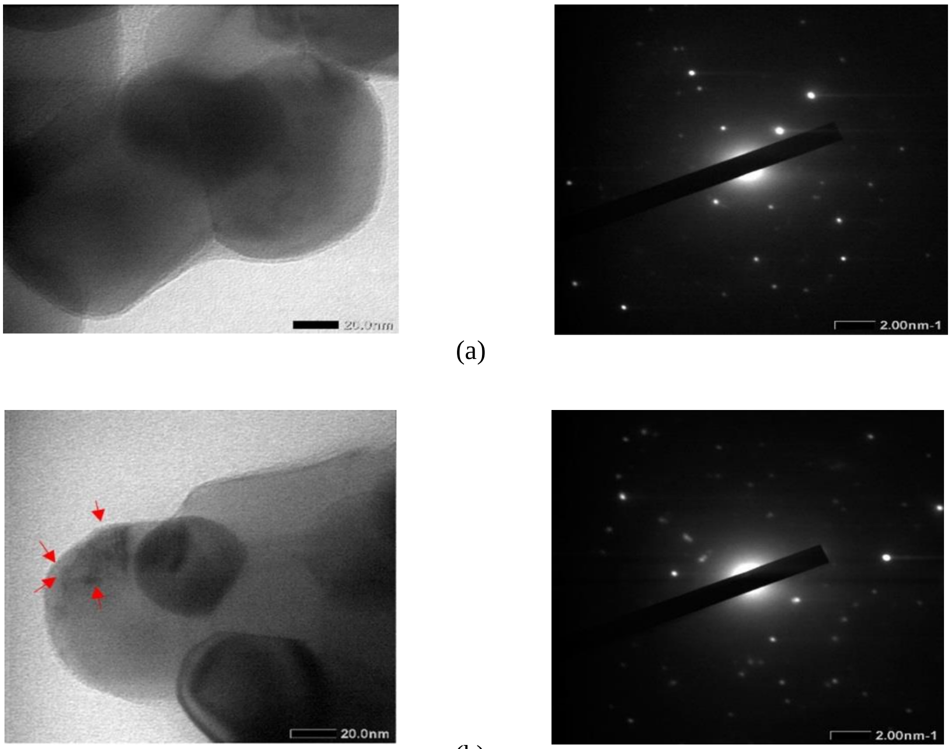

Fig. 3: TEM image and SAED of a) TiO_2 and b) TiO_2-Ag (2).

at the dark, since it needs light to release OH radicals that act as antibacterial.

Under visible light irradiation, TiO_2-Ag exhibits stronger antibacterial activity than TiO_2. TiO_2 that has high band gap energy, 3.2 eV for anatase and 3.0 for rutile, can not be activated by visible light due to the unsufficient energy of to stimulate electron release and OH radical formation. In contrast, TiO_2-Ag can be activated by visible light because it has lower band gap than TiO_2, that allows the visible light to stimulate electron release and produce OH radicals. The mutual effect of TiO_2 and Ag happened in the TiO_2-Ag so it has greater antibacterial activity.

The infulence of dose of TiO_2-Ag and irradiation time of visible light is also investigated. The chosen sample to investigate the dose and irradiation time was TiO_2-Ag (2), which has the greatest antibacterial activity under visible light. The result represented in Figs. 5 and 6.

At Fig. 5, increasing the doses of TiO_2-Ag can enhance the inhibition of the bacterial growth, but the further increase of TiO_2-Ag doses leads to slightly decrease in the inhibition. At low catalyst dose, only 30-34% reduction of *Staphylococcus aureus* cells is obtained. It can be attributed to the less availability of OH radicals compared to large amount of the bacteria cells. The increase

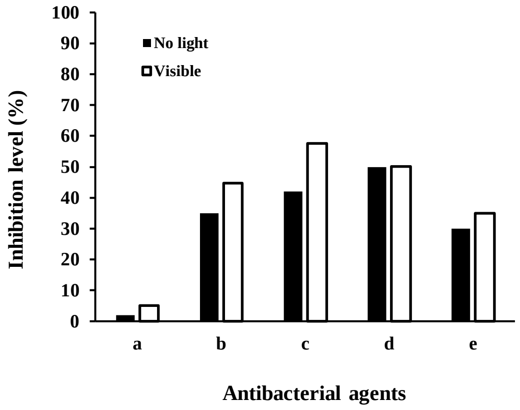

Fig. 4: The antibacterial activity of a) TiO_2, b) TiO_2-Ag(1), c) TiO_2-Ag(2), d) TiO_2-Ag(3) and e) TiO_2-Ag(4) under visible irradiation and dark condition.

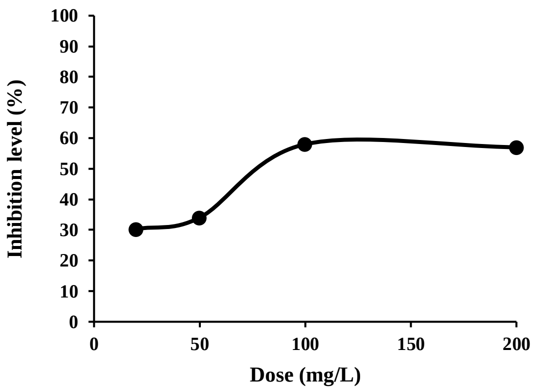

Fig. 5: The influence of TiO_2-Ag doses on the antibacterial activity.

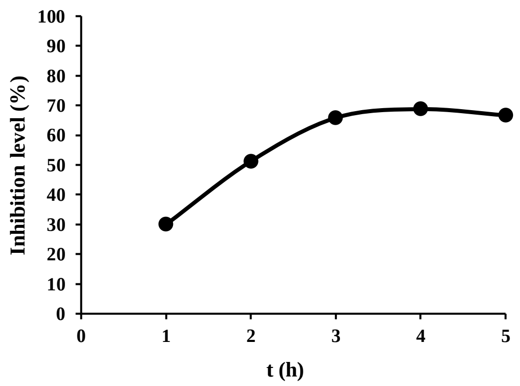

Fig. 6: The effect of irradiation time on the antibacterial activity of TiO_2-Ag).

in the dose of TiO_2-Ag shows the higher reduction of the bacteria cells. It implies the higher dose produce the sufficient amount of OH radicals to target the bacteria and leads to higher reduction of the bacteria in the medium. The higher dose of the catalyst loaded, the reduction of bacteria slightly decrease. It is caused by the large amount of TiO_2-Ag in the suspesion that increase the turbidity of the solution and blocks the radiation to cells and catalyst particle [17].

The effect of irradiation time of visible light on TiO_2-Ag is presented in Fig. 6. A general trend shows that the extention of the irradiation time can improve the antibacterial activity, but it keeps constant or slightly decreases when the irradiation time is further extended. The longer the light of exposure, the TiO_2-Ag might be saturated, that leads to no improvement in antibacterial activity.

The antibacterial agent was classified into bacteriocidal and bacteriostatic. Bacteriocidal agent conduct to kill the bacteria, while bacteriostatic only inhibit the growth of bacteria. In this study, the highest reduction of *Staphylococcus aures* cells under visible light irradiation is 58%. It was found that TiO_2-Ag is a bacteriostatic agent that inhibit the growth of the bacteria in the medium. The similar result has been reported that TiO_2-Ag only reduce 26% of *Staphylococcus aureus* cells in Muller-Hilton medium (11). The lower antimicrobial activity in medium could be attributed to the presence of dissolved organic matter, that could leads to decrease OH radicals amount in the medium.

CONCLUSIONS

Doping Ag on TiO_2 structure can shift its absorption into visible light irradiation. The absorption shift is found to depend on the amount of Ag doped. The highest absorption shift is demonstrated by 21.30 mg/g of Ag. It is also found obviously, the antibacterial activity of TiO_2-Ag is higher than TiO_2 under visible light irradiation. The highest antibacterial activity of TiO_2-Ag was achieved at 100 mg/L dose of TiO_2-Ag (21.30) under 3 h irradiation of visible light.

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REFERENCES

[1] Lubbers J.R., Chauhan S., Bianchine J.R., Controlled Clinical Evaluations of Chlorine Dioxide, Chlorite and Chlorate in Man, *Toxicol. Sci.*, **1(4)**: 334-338 (1981).

[2] Stübinger S., Sader R., Filippi A., The Use of Ozone in Dentistry and Maxillofacial Surgery: A Review. *Quintessence Int.*, **37(5)**:353–359 (2006).

[3] Fujishima A., Rao T.N., Tryk D.A., Titanium Dioxide Photocatalysis, *J. Photochem. Photobiol. C Photochem. Rev*, **1(1)**: 1–21 (2000).

[4] Chang S., Liu W. The Roles of Surface-doped Metal Ions (V, Mn, Fe, Cu, Ce, and W) in the Interfacial Behavior of TiO_2 Photocatalysts, *Appl. Catal. B Environ.*, **156–157**:466–75 (2014).

[5] Al-Hartomy O.A., Synthesis, Characterization, Photocatalytic and Photovoltaic Performance of Ag-doped TiO_2 Loaded on the Pt-carbon Spheres, *Mater. Sci. Semicond. Process.*, **27**(1):71-78 (2014).

[6] Silvestry-Rodriguez N., Sicairos-Ruelas E.E., Gerba C.P., Bright K.R., Silver as a Disinfectant, *Rev. Environ. Contam. Toxicol.*, **191**:23-45 (2007).

[7] Li Y., Ma M., Chen W., Li L., Zen M., Preparation of Ag-doped TiO_2 Nanoparticles by a Miniemulsion Method and Their Photoactivity in Visible Light Illuminations, *Mater. Chem. Phys.*, **129(1–2)**: 501–505 (2011).

[8] Chong M.N., Jin B., Chow C.W.K., Saint C., Recent Developments in Photocatalytic Water Treatment Technology: A Review, *Water Res.*, **44(10)**: 2997-3027 (2010).

[9] Wahyuni E.T., Roto R., Prameswari M., Antibacterial Activity of TiO_2-Ag-Nanoparticle under Visible Light, *Mater. Sci. Forum.*, **948**:33-42 (2019).

[10] Songkroah C., Nakbanpote W., Thiravetyan P., Recovery of Silver-thiosulphate Complexes with Chitin. *Process Biochem.*, **39(11)**:1553-1559 (2004).

[11] Naik K., Chatterjee A., Prakash H., Kowshik M., Mesoporous TiO_2 Nanoparticles Containing Ag Ion with Excellent Antimicrobial Activity at Remarkable Low Silver Concentrations, *J. Biomed. Nanotechnol.*, **9(4)**: 664-673 (2013).

[12] Tahir K., Ahmad A., Li B., Nazir S., Khan A.U., Nasir T., Khan Z.U.H., Naz R., Raza M., Visible Light Photo Catalytic Inactivation of Bacteria and Photo Degradation of Methylene Blue with Ag/TiO_2 Nanocomposite Prepared by a Novel Method, *J. Photochem. Photobiol. B Biol.*, **162**:189-98 (2016)

[13] Chan S.C., Barteau M.A., Preparation of Highly Uniform Ag/TiO_2 and Au/TiO_2 Supported Nanoparticle Catalysts by Photodeposition, *Langmuir*, **21(12)**: 5588–5595 (2005).

[14] Ko S., Banerjee C.K., Sankar J., Photochemical Synthesis and Photocatalytic Activity in Simulated Solar Light of Nanosized Ag Doped TiO_2 Nanoparticle Composite, *Compos. Part B Eng.*, **42(3)**: 579–583 (2011).

[15] Wahyuni E.T., Roto R., Novarita D., Suwondo K.P., Kuswandi B., Preparation of TiO_2/AgNPs by Photodeposition Method Using Ag(I) Present in Radiophotography Wastewater and Their Antibacterial Activity in Visible Light Illumination, *J. Environ. Chem. Eng.*, **7(4)**: 103178 (2019).

[16] Sobana N., Selvam K., Swaminathan M., Optimization of Photocatalytic Degradation Conditions of Direct Red 23 Using Nano-Ag Doped TiO_2, *Sep. Purif. Technol.*, **62(3)**: 648–653 (2008).

[17] Li H., Cui Q., Feng B., Wang J., Lu X., Weng J., Antibacterial Activity of TiO_2 Nanotubes: Influence of Crystal Phase, Morphology and Ag Deposition, *Appl. Surf. Sci.*, **284**: 179-183 (2013).