Efficient Destruction of Pathogenic Bacteria with NiO/SrBi₂O₄ under Visible Light Irradiation

CHUN HU,* XUEXIANG HU,
JIAN GUO, AND JIUHUI QU*

State Key Laboratory of Environmental Aquatic Chemistry,
Research Center for Eco-Environmental Sciences, Chinese
Academy of Sciences, Beijing 100085, China

The photocatalytic inactivation of pathogenic bacteria in water was investigated systematically with NiO/SrBi₂O₄ under visible light ($\lambda > 420$ nm) irradiation. The catalyst was found to be highly effective in killing *Escherichia coli*, a Gram-negative bacterium, and *Staphylococcus aureus*, a Gram-positive bacterium. ESR studies revealed that $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ were involved as the active species in the photocatalytic reaction. The decomposition process of the cell wall and the cell membrane was directly observed by TEM and further confirmed by the determination of potassium ion (K^+) leakage from the killed bacteria. A possible cell damage mechanism by visible-light-driven NiO/SrBi₂O₄ is proposed. In addition, the effects of pH, methanol, and inorganic ions on bacterial photocatalytic inactivation were investigated. These results indicated that the electrostatic force interaction of bacteria-catalyst is crucial for high bactericidal efficiency.

Introduction

Disinfectants, in addition to effectively killing harmful microorganisms, are powerful oxidants that oxidize the organic matter and bromide naturally present in most source waters, forming disinfection byproducts (DBPs). Chlorine, ozone, chlorine dioxide, and chloramines are the most common disinfectants in use, and each produces its own suite of chemical DBPs in finished drinking water (1). Pathogenic organisms provide the primary human health risk from drinking water, while chemical DBPs also provide an unintended health hazard. Therefore, the development of more effective sterilization technologies has become an urgent issue.

In 1985, Matsunaga and co-workers reported for the first time that TiO₂ photocatalyst could kill bacterial cells in water (2). Since then, numerous studies related to the bactericidal effect of TiO₂ photocatalyst has been reported (3–6). TiO₂ photocatalytic disinfection seems to be a promising technique. However, TiO₂ only absorbs wavelengths in the near-UV region ($\lambda < 400$ nm), which is about 3% of the solar spectrum. Therefore, TiO₂ cannot efficiently utilize the visible light of solar energy for photocatalytic disinfection. The development of efficient visible-light-driven photocatalysts for photocatalytic disinfection has been a crucial issue from the viewpoint of using solar energy. The pioneering work done by Zou et al. (7) introduced water splitting for H₂ and O₂ evolution in a stoichiometric amount over the NiO_x/In_{0.9}Ni_{0.1}TaO₄ photocatalyst under visible light irradiation. Fol-

lowing this work, many new visible-light-driven catalysts have also been reported (8–13). However, only a few studies (8, 9) have been conducted so far for the elimination of organic pollutants in water. Moreover, in the process of pollutant degradation, there was no direct evidence to indicate whether these catalysts excited by visible light could create electron–hole pairs and what reactive oxygen species (ROS) would be involved. To date, the ROS have not been determined directly by electron spin resonance (ESR), which is an effective method in identification of active radicals (14). A visible-light-induced degradation mechanism was conjectured based on other indirect data and the energy band theory of oxide semiconductors.

Recently, we have synthesized SrBi₂O₄ powders by a coprecipitation method, and NiO was loaded on the powder by impregnation. In the present study, for the first time, the NiO/SrBi₂O₄ photocatalytic disinfection of pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* in water under visible light irradiation was studied in detail from the mechanistic and kinetic viewpoints. ESR was used to detect the reactive species involved in the reactions. NiO/SrBi₂O₄ was found to be a novel visible-light-driven bactericidal catalyst.

Experimental Section

Materials. The reagent 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), used as the spin trapping agent in the ESR studies, was purchased from the Sigma Chemical Co. and stored at -20 °C in a freezer. *Escherichia coli* (*E. coli* DH 4 α) and *Staphylococcus aureus* (*S. aureus* ATCC 6538) were purchased from the Institute of Microbiology, Chinese Academy of Sciences. All other chemicals were analytical grade. Deionized and doubly distilled water was used throughout this study.

Preparation of Photocatalysts. Stoichiometric amounts of Sr(NO₃)₂ and Bi(NO₃)₃·5H₂O were dissolved in deionized water, and a solution of ethylene diamine tetraacetic acid in ammonia and citric acid were added to the above aqueous solution. The pH value was adjusted to 4.0 by ammonia. During this process, the mixed solution was stirred continuously until a white precipitate was formed. Then, the precipitate was dried at 160 °C to remove water completely, calcined at 350 °C for 10 h, and finally calcined at 750 °C in a muffle furnace for 12 h in air. Subsequently, 2 g of the as-synthesized sample was put into in 4 mL of 0.1 M Ni-(NO₃)₂ aqueous solution and was evaporated at 70 °C by magnetic stirring. Then the dried powder was calcined at 300 °C for 1 h.

Characterization. Powder X-ray diffraction of NiO/SrBi₂O₄ was recorded on a Scintag-XDS-2000 diffractometer with Cu K α radiation ($\lambda = 1.54059$ Å). UV–visible absorption spectra of the samples were recorded on a UV–vis spectrophotometer (Hitachi UV-3100) with an integrating sphere attachment. The analyzed range was 200–800 nm, and BaSO₄ was the reflectance standard. The XPS data were taken on an AXIS-Ultra instrument from Kratos using monochromatic Al K α radiation (225 W, 15 mA, 15 kV) and low-energy electron flooding for charge compensation. To compensate for surface charges effects, binding energies were calibrated using the C1s hydrocarbon peak at 284.80 eV. Electron spin resonance (ESR) spectra were obtained using a Bruker model ESP 300 E electron paramagnetic resonance spectrometer equipped with a quanta-Ray Nd:YAG laser system as the irradiation light source ($\lambda = 532$ nm). The settings were center field, 3480.00 G; microwave frequency, 9.79 GHz; and power, 5.05 mW. Determination of the concentration of H₂O₂ formed in the catalyst aqueous suspension under visible light irradiation

* Corresponding author phone: +86-10-62849171; fax: +86-10-62923541; e-mail: huchun@rcees.ac.cn (Hu C); jhqu@rcees.ac.cn (Qu J).

FIGURE 1. XRD pattern of NiO/SrBi₂O₄. The inset shows UV–vis diffuse reflectance spectra of SrBi₂O₄ and NiO/SrBi₂O₄.

was performed with a photometric method described in the literature (15). The zeta-potential of catalysts in KNO₃ (10^{−3} M) solution and bacteria cells in NaCl (0.1 M) solution was measured with a Zetasizer 2000 (Malvern Co., United Kingdom). Every reading of the instrument was recorded after three consistent readings were attained.

Bactericidal Activity. Two types of bacteria, *S. aureus*, a Gram-positive bacterium, and *E. coli*, a Gram-negative bacterium, were used as model bacteria in this study. They were incubated in Luria–Bertani (LB) nutrient solution at 37 °C for 18 h with shaking and then washed by centrifuging at 4000 rpm. The treated cells were then resuspended and diluted to $\sim 1 \times 10^8$ (8) colony forming units (cfu/mL) with 0.9% saline. All materials used in the experiments were autoclaved at 121 °C for 25 min to ensure sterility. The diluted cell suspension and photocatalyst were added to a 100-mL beaker with a cover. The final photocatalyst concentration was adjusted to 0.2 g/L, and the final bacterial cell concentration was $\sim 8 \times 10^7$ (7) cfu/mL. The reaction volume was 30 mL. The reaction mixture was stirred with a magnetic stirrer throughout the experiment. The light source for photocatalysis was a 350-W Xe arc lamp (Shanghai Photoelectron Device Ltd.). Light passed through a water filter and a UV cutoff filter ($\lambda > 420$ nm) and then was focused onto the beaker reactor. The intensity of the illumination was 2.8 mW/cm². The reaction temperature was maintained at 25 °C. The initial solution pH was adjusted by diluted aqueous solutions of NaOH or HCl, and the solution pH varied by less than 1 pH unit throughout the reaction. A bacterial suspension without photocatalyst was irradiated as a control, and the reaction mixture with no visible light irradiation was used as a dark control. Before and during the light irradiation, an aliquot of the reaction solution was immediately diluted with saline, an appropriate dilution of the sample was incubated at 37 °C for 24 h on a nutrient agar medium, and then the colonies were counted to determine the number of viable cells. For the measurement of K⁺ leakage from the inactive bacteria, at every time interval, 1 mL of the illuminated bacterial suspension was centrifuged, and the supernatant was used for inductively coupled plasma optical emission spectrometry (ICP-OES, OPTIMA 2000, Perkin-Elmer Co.). All of the above experiments were repeated three times.

Results and Discussion

Characterization of NiO/SrBi₂O₄. Figure 1 shows the XRD patterns of NiO/SrBi₂O₄. The monoclinic structure SrBi₂O₄ is the predominant phase, with less Sr_{2.25}Bi_{6.75}O_{12.38} phase. No XRD diffraction peaks of oxide nickel species were observed. This presumably contributed to the incorporation of its low content, small particle size, and high dispersion on the surface of SrBi₂O₄. The crystallite size of NiO/SrBi₂O₄ was

FIGURE 2. Temporal course of the *E. coli* inactivation ($\sim 8 \times 10^7$ cfu/mL, 30 mL, pH=7) in aqueous dispersions containing 0.2 g/L of catalysts under visible light irradiation: (a) no catalyst, (b) NiO/SrBi₂O₄ in the dark, (c) SrBi₂O₄, and (d) NiO/SrBi₂O₄.

181 nm as calculated with the Debye–Scherrer equation from the line width of the XRD data. The specific surface area of the sample was 1.77 m²/g. Determination of the oxidation state of the doped nickel was carried out by measuring Ni2p_{3/2} binding energy (BE) with XPS. The BE 854.0 was assigned to NiO. The concentration of Ni in the surface of the catalyst was ~ 5.54 atom % according to the XPS data (XPS sensitivity factor: 4.04), while based on the dosages of Ni(NO₃)₂ and SrBi₂O₄, the concentration of Ni in SrBi₂O₄ was ~ 1.40 atom %, lower than the experimental value. This indicates that most NiO was supported on the surface of SrBi₂O₄. The optical absorption spectra of SrBi₂O₄ showed a visible light absorption band of 400–540 nm, as depicted in the inset of Figure 1. With the loading of NiO, the NiO/SrBi₂O₄ displayed the same absorption edge as SrBi₂O₄. However, the NiO/SrBi₂O₄ specimen exhibited a greater light attenuation throughout the visible wavelengths consistent with the grayed color of the catalyst.

Bacterial Inactivation under Visible Light Irradiation. The bactericidal activities of the samples were evaluated by the inactivation of *E. coli* in water under visible light irradiation. As shown in Figure 2, 7.5 log *E. coli* was almost completely killed within 50 min in NiO/SrBi₂O₄ suspension under visible light irradiation. Neither visible light nor NiO/SrBi₂O₄ in the dark showed any bactericidal effects on *E. coli*, indicating that the photocatalyst itself is not toxic to *E. coli*. SrBi₂O₄ showed some visible light bactericidal activity; 0.24 log cells were inactivated. The results indicated that the photocatalytic activity of SrBi₂O₄ was greatly enhanced by loading NiO on the external surface of SrBi₂O₄. As suggested earlier (16), the mechanism of photocatalytic reactions involves the generation of the intermediate H₂O₂. The formation of H₂O₂ was determined during the inactivation process of *E. coli* in aqueous SrBi₂O₄ and NiO/SrBi₂O₄ dispersions, respectively (Figure S1, Supporting Information). In the *E. coli*–NiO/SrBi₂O₄ system, the quantities of H₂O₂ increased with irradiation time, then decreased continuously, and finally increased again, reaching a maximal value of 4.7×10^{-6} M. During the photocatalytic reaction, the formed H₂O₂ was consumed by further reacting with other active species and *E. coli*. The formation and decomposition of H₂O₂ possibly occurred at the same time. Therefore, the concentration of H₂O₂ displayed disorderly change. Similarly, in the *E. coli*–SrBi₂O₄, the concentration of H₂O₂ first increased with irradiation time, reaching a maximal value of 7×10^{-7} M, then decreased, and then increased again. Whether the formation rate or the maximal quantity of H₂O₂ formed is chosen, it is clear that the NiO/SrBi₂O₄ is significantly more active than the SrBi₂O₄. According to the

FIGURE 3. DMPO spin-trapping ESR spectra recorded at ambient temperature in methanol dispersion (for $\text{DMPO-O}_2^{\bullet-}$, A) and aqueous dispersion (for DMPO-OH , B) under irradiation of 532 nm: (a) SrBi_2O_4 and (b) $\text{NiO/SrBi}_2\text{O}_4$ before and after irradiation.

photocatalytic mechanism (17, 18), the H_2O_2 could be formed from these reactions of photogenerated electrons and holes with adsorbed oxygen/water, where $\text{O}_2^{\bullet-}$ and $\cdot\text{OH}$ were possibly involved. Therefore, the results indicated that $\text{O}_2^{\bullet-}$ and $\cdot\text{OH}$ reactive species were possibly produced in aqueous $\text{NiO/SrBi}_2\text{O}_4$ or SrBi_2O_4 suspensions under visible light irradiation. To confirm this conjecture, the ESR spin-trap technique (with DMPO) was used to obtain information on the active radicals involved (Figure 3). Since $\text{O}_2^{\bullet-}$ radicals in water are very unstable and undergo facile disproportionation rather than slow reaction with DMPO (19), the involvement of $\text{O}_2^{\bullet-}$ radicals in $\text{NiO/SrBi}_2\text{O}_4$ and SrBi_2O_4 systems was examined respectively in methanol using a Nd:YAG laser (532 nm) as the irradiation source (Figure 3A). The six characteristic peaks of the $\text{DMPO-O}_2^{\bullet-}$ adducts were observed only in $\text{NiO/SrBi}_2\text{O}_4$ dispersion under visible light irradiation. No $\text{O}_2^{\bullet-}$ signals were detected in SrBi_2O_4 systems under otherwise identical conditions. Similarly, the DMPO-OH species were detected successfully in aqueous $\text{NiO/SrBi}_2\text{O}_4$ dispersion under visible light irradiation (Figure 3B). Four characteristic peaks of DMPO-OH , 1:2:2:1 quartet pattern, were observed. No such signals were detected in the dark and visible-light-irradiated SrBi_2O_4 system. The evidence that $\cdot\text{OH}$ and $\text{O}_2^{\bullet-}$ are produced on the surface of visible illuminated $\text{NiO/SrBi}_2\text{O}_4$ provides a solid indication that the catalyst can be efficiently excited by visible light to create electron-hole pairs and that the charge separation is maintained long enough to react with adsorbed oxygen/ H_2O and to produce a series of active oxygen radicals which finally induces the decomposition of pollutants and bacteria (20). Neither $\cdot\text{OH}$ nor $\text{O}_2^{\bullet-}$ was formed in the visible-light-illuminated SrBi_2O_4

FIGURE 4. Survival of *S. aureus* ($\sim 8 \times 10^7$ cfu/mL, 30 mL, pH=7) in different concentrations of $\text{NiO/SrBi}_2\text{O}_4$ dispersion under visible light irradiation: (a) control 1 g/L catalyst in the dark, (b) 0.2 g/L, (c) 0.5 g/L, and (d) 1 g/L.

FIGURE 5. (a) Zeta potentials for a suspension 1.0 g/L of $\text{NiO/SrBi}_2\text{O}_4$ in the presence of KNO_3 (10^{-3} M), and (b) and (c) zeta potentials for suspensions of *E. coli* and *S. aureus* in the presence of NaCl (0.1 M).

FIGURE 6. Survival of *E. coli* with visible-light-illuminated $\text{NiO/SrBi}_2\text{O}_4$ dispersions (0.2 g/L, pH=7) under otherwise different conditions: (a) 0.1 M methanol with no catalyst, (b) 0.1 M NaHCO_3 , (c) 0.1 M K_2HPO_4 , (d) 0.1 M Na_2SO_4 , (e) 0.1 M methanol, and (f) only illuminated $\text{NiO/SrBi}_2\text{O}_4$.

suspension, indicating that the recombination rate of the photogenerated electron-hole pairs from the SrBi_2O_4 excited by visible light was higher than their separation rate. The

FIGURE 7. (A) Untreated *E. coli* and (B) and (C) TEM micrographs of *E. coli* in visible-light-illuminated NiO/SrBi₂O₄ suspension for 120 min.

results demonstrated that NiO on the external surface of SrBi₂O₄ trapped photogenerated electrons, enhancing the electron–hole separation leading to active species formation, which agreed with previous findings (21).

Interaction of Bacteria–NiO/SrBi₂O₄. *S. aureus* was also killed efficiently in aqueous NiO/SrBi₂O₄ suspensions under visible light irradiation (Figure 4). In the presence of 1 g/L of NiO/SrBi₂O₄, complete *S. aureus* inactivation of 7.2 log occurred at 80 min of irradiation, whereas no significant *S. aureus* inactivation was observed in the dark. However, when the concentration of NiO/SrBi₂O₄ was 0.2 g/L, only 1 log *S. aureus* was inactivated at 60 min, while 7.5 log *E. coli* were completely removed under otherwise identical conditions. Clearly, NiO/SrBi₂O₄ shows much higher bactericidal activity for *E. coli* than *S. aureus*. The results mainly revealed the interaction of bacteria and NiO/SrBi₂O₄. The charges of the bacteria and NiO/SrBi₂O₄ under different pH conditions are shown in Figure 5. In the range of pH 2–9, the surface of the catalyst is positively charged, whereas the overall charges of *E. coli* and *S. aureus* are negative, and the zeta-potential of *E. coli* is more negative than that of *S. aureus* in the range of pH = 4–9. Therefore, under the same pH conditions, the role of electrostatic attraction between *E. coli* and the catalyst is stronger than that one of *S. aureus* and the catalyst, leading to more *E. coli* adsorption onto the surface of the catalyst. Thus, the catalyst exhibits more activity for the killing of *E. coli*. Hamouda and co-workers (22) have also shown if the antimicrobial composition had the same charge as the bacteria cells, this induced repulsion and prevented contact, while the addition of EDTA/Tris buffer to the formulation changed the charge and considerably improved the activity of the formulation. To verify this point, the effect of pH on bacteria photocatalytic inactivation was carried out. The rate of *S. aureus* inactivation did not significantly change because the zeta-potential of *S. aureus* (from Figure 4) was almost unchanged with increasing pH (Figure S2A, Supporting Information). In contrast, pH exhibited great influence on the *E. coli* inactivation. At pH = 3.86, the inactivation of *E.*

coli became lower; only 1.97 log *E. coli* inactivation occurred at 60 min irradiation (Figure S2B, Supporting Information). The zeta-potential of *E. coli* tended to be less negative at pH = 3.86, resulting in less *E. coli* being tightly bound with the catalyst surface. Thus, the inactivation of *E. coli* was inhibited. When the zeta-potential of *E. coli* was very negative at pH = 7, the electrostatic attraction between *E. coli* and the catalyst became stronger, leading to a higher inactivation rate. At pH = 8.94, the zeta-potentials of the catalyst and *E. coli* did not change much compared with the condition of pH = 7, so the inactivation rate of *E. coli* was similar to that at pH = 7.

Furthermore, Figure 6 shows the effect of methanol on the inactivation of *E. coli*. Methanol with no catalyst hardly showed any bactericidal effects on *E. coli* under visible light irradiation (curve a), indicating methanol itself at the tested concentration is not toxic to *E. coli*. In comparison, in only visible-light-illuminated NiO/SrBi₂O₄ (curve f) the addition of methanol did not greatly affect the *E. coli* killing (curve e). As is well-known, methanol has been found to both scavenge holes and react with •OH (23). However, there was not a significant amount of methanol adsorbed on the catalyst in aqueous systems, and the interaction of electrostatic force between *E. coli* and the catalyst was not changed by the addition of methanol. In addition, methanol does not react with O₂^{•−}, as shown by the fact that O₂^{•−} could be determined in methanol by the ESR method (20). O₂^{•−} radicals were still reactive species in the methanol/*E. coli*-catalyst system, causing the inactivation of *E. coli*. Figure 6 also shows the effect of different ions on the bactericidal inactivation under visible-light-illuminated NiO/SrBi₂O₄. The bactericidal activity of NiO/SrBi₂O₄ was markedly inhibited by the addition of ions. In 0.1 M Na₂SO₄ (curve d), 1.4 log *E. coli* was killed, whereas less than 1 log *E. coli* was killed in the presence of 0.1 M NaHCO₃ and K₂HPO₄ (curves b and c). These ions might play two roles in the photocatalytic inactivation of *E. coli*. The first one was that their high adsorption on NiO/SrBi₂O₄ caused the formation of a negatively charged layer on the surface of the catalyst, which repulsed bacteria,

resulting in a slower disinfection rate. Another was that these adsorbed ions reacted with h^+ and $\cdot\text{OH}$ to form $\text{SO}_4^{\cdot-}$, $\text{HPO}_4^{\cdot-}$, and $\text{HCO}_3^{\cdot-}$, which are less reactive than $\cdot\text{OH}$ and h^+ . ESR measurement confirmed this. No signals of $\text{DMPO}\cdot\text{OH}$ were detected in visible-light-irradiated $\text{NiO}/\text{SrBi}_2\text{O}_4$ with 0.1 M Na_2SO_4 or K_2HPO_4 (Figure S3, Supporting Information). The same phenomena also were observed in the presence of 0.1 M NaHCO_3 under otherwise identical conditions. The results indicated that reactive species and the electrostatic attractive interaction of bacteria-catalyst are important factors for high bactericidal activity.

Cell Damage Mechanism. To understand the bactericidal mechanism of various reactive species (e.g., $\cdot\text{OH}$, $\text{O}_2^{\cdot-}$, and H_2O_2), the morphology of bacteria at different stages during bactericidal experiments was checked by the TEM study. Figure 7A shows the TEM images of *E. coli*. Characteristics of the bacteria are a well-defined cell wall as well as the evenly rendered interior of the cell, which corresponds to the presence of proteins and DNA. Great changes had taken place to the morphology of *E. coli* that had been illuminated for 120 min (Figure 7B,C). The cell wall was decomposed, and the rendered interior of the cell became white, indicating that the outer membrane of the cell was damaged leading to a leakage of the interior component. Similarly, the cell wall of *S. aureus* was also destructed resulting in a leakage of the interior component (Figure S4, Supporting Information). Based on the TEM investigation, the cell wall, the peptidoglycan layer, and the cell membrane of the bacteria were decomposed by reactive species. K^+ exists universally in bacteria (24) and plays a role in the regulation of polysome content and protein synthesis. Therefore, K^+ leakage from the inactivated bacteria was used in this work to examine the permeability of the cell membrane (Figure S5, Supporting Information). In both the dark and visible light irradiation with no catalyst conditions (control experiments), there was nearly the same K^+ leakage from *E. coli* cells. The K^+ concentration gradually increased and became approximately steady with increasing reaction time. In the visible-light-illuminated $\text{NiO}/\text{SrBi}_2\text{O}_4$ suspensions, K^+ immediately leaked out, and the leakage increased in parallel with the inactivation of *E. coli* with irradiation time, and the resultant K^+ concentrations were much higher than those in the control experiments. The results implied that the visible-light-induced photocatalytic reaction caused a change in the cell membrane permeability and the resultant leakage of intracellular substances. In the meanwhile, much higher concentrations of K^+ were released by the inactivation of the *S. aureus* cells in the visible-light-illuminated $\text{NiO}/\text{SrBi}_2\text{O}_4$ suspensions compared to the K^+ concentrations from the *S. aureus* cells under the control conditions. These results demonstrate that the K^+ leakage was consistent with the decomposition of the cell wall and the cell membrane. According to previous research (3, 25), possible mechanisms for the bactericidal effect of TiO_2 photocatalysis have been proposed: UV illumination of TiO_2 produces various reactive species, causing disruption of the cell membrane and the cell wall and the resultant leakage of intracellular molecules. Based on all of the above information, we may deduce that the visible light bactericidal mechanism of $\text{NiO}/\text{SrBi}_2\text{O}_4$ is similar to that of TiO_2 in UV illumination. The cell wall and the cell membrane were subsequently decomposed by various reactive species (e.g., $\cdot\text{OH}$, $\text{O}_2^{\cdot-}$, H_2O_2), causing cell death.

Acknowledgments

This work was supported by the Natural Sciences Foundation of China (Grant Nos. 20537020, 20577062, 20377050, and 50538090) and the National 863 Project of China (Grant No. 2005AA642030). We thank Dr. Chuncheng Chen (Institute of Chemistry, Chinese Academy of Sciences) for his valuable discussion about ESR studies.

Supporting Information Available

Results for the formation of H_2O_2 , inactivation of *S. aureus* and *E. coli* under different pH conditions, DMPO spin-trapping ESR spectra in the presence of different ions, and TEM of *S. aureus*. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Literature Cited

- Richardson, S. D. Disinfection by-products and other emerging contaminants in drinking water. *Trends Anal. Chem.* **2003**, *22*, 666–684.
- Matsunaga, T.; Tomada, R.; Nakajima, T.; Wake, H. Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiol. Lett.* **1985**, *29*, 211–214.
- Jacoby, W. A.; Maness, C. P.; Wolfrum, E. J.; Blake, D. M.; Fennel, J. A. Mineralization of bacterial cell mass on a photocatalytic surface in air. *Environ. Sci. Technol.* **1998**, *32*, 2650–2653.
- Linkous, A. C.; Carter, G. J.; Locuson, D. B.; Ouellette, A. J.; Slattery, D. K.; Simitha, L. A. Photocatalytic inhibition of algae growth using TiO_2 , WO_3 , and cocatalyst modifications. *Environ. Sci. Technol.* **2000**, *34*, 4754–4758.
- Sunada, K.; Kikuchi, Y.; Hashimoto, K.; Fujishima, A. Bactericidal and detoxification effects of TiO_2 thin film photocatalysts. *Environ. Sci. Technol.* **1998**, *32*, 726–728.
- Yu, J. C.; Ho, W. K.; Yu, J.; Yin, H.; Wong, P. K.; Zhao, J. Efficient visible-light-induced photocatalytic disinfection on sulfur-doped nanocrystalline titania. *Environ. Sci. Technol.* **2003**, *39*, 1175–1179.
- Zou, Z.; Ye, J.; Sayama, K.; Arakawa, H. Direct splitting of water under visible light irradiation with an oxide semiconductor photocatalyst. *Nature* **2001**, 625–627.
- Fu, H.; Pan, C.; Yao, W.; Zhu, Y. Visible-light-induced degradation of Rhodamine B by nanosized Bi_2WO_6 . *J. Phys. Chem. B* **2005**, *109*, 22432–22439.
- Tang, J.; Zou, Z.; Ye, J. Efficient photocatalytic decomposition of organic contaminants over CaBi_2O_4 under visible-light irradiation. *Angew. Chem., Int. Ed.* **2004**, *116*, 4563–4566.
- Ye, J.; Zou, Z.; Oshikiri, M.; Matsushita, A.; Shimoda, M.; Imai, M.; Shishido, T. A novel hydrogen-evolving photocatalyst InVO_4 active under visible light irradiation. *Chem. Phys. Lett.* **2002**, *356*, 221–226.
- Tang, J.; Zou, Z.; Ye, J. Photophysical and photocatalytic properties of AgInW_2O_8 . *J. Phys. Chem. B* **2003**, *107*, 14265–14269.
- Kim, H.; Hwang, D.; Lee, J. An undoped, single-phase oxide photocatalyst working under visible light. *J. Am. Chem. Soc.* **2004**, *126*, 8912–8913.
- Ye, J.; Zou, Z.; Matsushita, A. A novel series of water splitting photocatalyst NiM_2O_6 ($\text{M} = \text{Nb}, \text{Ta}$) active under visible light. *Int. J. Hydrogen Energy* **2003**, *28*, 651–655.
- Zhao, J.; Wu, T.; Wu, K.; Hidaka, H.; Serpon, N. Photoassisted degradation of dye pollutants. 3. Degradation of the cationic dye Rhodamine B in aqueous anionic surfactant/ TiO_2 dispersions under visible light irradiation: evidence for the need of substrate adsorption on TiO_2 particles. *Environ. Sci. Technol.* **1998**, *32*, 2394–2400.
- Bader, H.; Sturzenegger, V.; Hoigne, J. Photometric method for the determination of low concentrations of hydrogen peroxide by the peroxidase catalyzed oxidation of N, N-diethyl-p-phenylenediamine (DPD). *Water Res.* **1988**, *22*, 1109–1115.
- Shiraishi, F.; Nakasako, T.; Hua, Z. Formation of hydrogen peroxide in photocatalytic reactions. *J. Phys. Chem. A* **2003**, *107*, 11072–11081.
- Mills, A.; Hunte, S. L. An overview of semiconductor photocatalysis. *J. Photochem. Photobiol., A* **1997**, *108*, 1–35.
- Hoffman, M. R.; Martin, S. T.; Choi, W.; Bahnemann, D. W. Environmental applications of semiconductor photocatalysis. *Chem. Rev.* **1995**, *95*, 69–96.
- Ma, W.; Huang, Y.; Li, J.; Chen, M.; Song, W.; Zhao, J. An efficient approach for the photodegradation of organic pollutants by immobilized iron ions at neutral pHs. *Chem. Commun.* **2003**, 1582–1583.
- Linsebigler, A. L.; Lu, G.; Yates, J. T. Photocatalysis on TiO_2 surfaces: principles, mechanisms, and selected results. *Chem. Rev.* **1995**, *95*, 735 and references therein.
- Zou, Z.; Ye, J.; Arakawa, H. Surface characterization of nanoparticles of $\text{NiO}_x/\text{In}_0.9\text{Ni}_0.1\text{TaO}_4$: Effects on photocatalytic activity. *J. Phys. Chem. B* **2002**, *106*, 13098–13101.

- (22) Hamouda, T.; Baker, J. R., Jr. Antimicrobial mechanism of action of surfactant lipid preparations in enteric Gram-negative bacilli. *J. Appl. Microbiol.* **2000**, *89*, 397–403.
- (23) Ulmann, M.; Augustynky, J. The involvement of surface-bonded intermediates in the competitive photo-oxidation of methanol and hydroxyl ions at a TiO₂ electrode. *Chem. Phys. Lett.* **1987**, *141*, 154–158.
- (24) Saito, T.; Iwase, T.; Morioka, T. Mode of photocatalytic bactericidal action of powdered semiconductor TiO₂ on mutant streptococci. *J. Photochem. Photobiol., B* **1992**, *14*, 369–379.
- (25) Maness, C. P.; Smolinski, S.; Blake, D. M.; Huang, Z.; Wolfrum, E. J.; Jacoby, W. A. Bactericidal activity of photocatalytic TiO₂ reaction: Toward an understanding of its killing mechanism, *Appl. Environ. Microbiol.* **1999**, *65*, 4094–4098.

Received for review November 30, 2005. Revised manuscript received June 23, 2006. Accepted July 11, 2006.

ES052405V