Synthesis of Magnetically Separable Ag₃PO₄/TiO₂/Fe₃O₄ Heterostructure with Enhanced Photocatalytic Performance under Visible Light for Photoinactivation of Bacteria

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ABSTRACT: Silver orthophosphate (Ag₃PO₄) is a low-band-gap photocatalyst that has received considerable research interest in recent years. In this work, the magnetic Ag₃PO₄/TiO₂/Fe₃O₄ heterostructured nanocomposite was synthesized. The nanocomposite was found to exhibit markedly enhanced photocatalytic activity, cycling stability, and long-term durability in the photodegradation of acid orange 7 (AO7) under visible light. Moreover, the antibacterial film prepared from Ag₃PO₄/TiO₂/Fe₃O₄ nanocomposite presented excellent bactericidal activity and recyclability toward Escherichia coli (E. coli) cells under visible-light irradiation. In addition to the intrinsic cytotoxicity of silver ions, the elevated bactericidal efficiency of Ag₃PO₄/TiO₂ can be largely attributed to its highly enhanced photocatalytic activity. The photogenerated hydroxyl radicals and superoxide ions on the formed Ag/Ag₃PO₄/TiO₂ interfaces cause considerable morphological changes in the microorganism’s cells and lead to the death of the bacteria.

KEYWORDS: photocatalyst, heterostructure, visible light, photoinactivation, bactericidal activity, recyclability

1. INTRODUCTION
Photocatalysts, such as TiO₂,¹² ZnO,³ and Mo₃O₉,⁴ can be used as antimicrobial materials in the killing or growth inhibition of bacteria. As one well-known component, titanium dioxide (TiO₂) has been extensively studied since Fujishima and Honda demonstrated its high photocatalytic activity.⁵ TiO₂ shows great prospects as a photocatalyst in decomposing organic pollutants in the environment, as a self-cleaning coating for buildings, and also as an antimicrobial material.⁶−¹⁰ When TiO₂ is irradiated with light with an energy equal to or greater than its band-gap energy, electron−hole pairs are generated in the photocatalyst and subsequently react with H₂O and O₂ molecules to produce highly reactive hydroxyl radicals and superoxide ions, respectively, at the catalyst/water interface. The resulting active oxygen species can oxidize and damage the cells of microorganisms at the surface of the catalyst.¹¹ However, because of its wide band gap (E_g ≈ 3.0−3.2 eV), TiO₂ is particularly suitable for applications based on UV-light irradiation. Additionally, the practical application of TiO₂ is also limited by its insufficient electron−hole separation, which leads to a lower energy conversion.¹²

Very recently, a breakthrough was made by Yi et al., who reported the use of silver orthophosphate (Ag₃PO₄) to oxidize water and decompose organic contaminants in aqueous solutions by visible-light irradiation.¹³ Ag₃PO₄ has a relatively narrow band gap (2.36−2.43 eV) and is thus active under visible-light irradiation (λ < 530 nm). Therefore, as a highly efficient photocatalyst, Ag₃PO₄ could behave as a potential antimicrobial material and could have promise in various antimicrobial applications.¹⁴,¹⁵ Furthermore, silver and silver-based compounds are well-known antimicrobial biomaterials because of their low toxicity for mammalian cells and excellent broad-spectrum antimicrobial activity.¹⁶−²¹ However, Ag₃PO₄ is prone to photoreduction and decomposition if no sacrificial reagent is supplied.²²,²³ To overcome this problem, many studies have focused on heterocoupling,²⁴,²⁵ fabricating signal crystals,²⁶ decreasing the particle size,²⁷ and synthesizing Ag/Ag₃PO₄ plasmonic nanocomposites.²⁸,²⁹ As the valence band (VB) level of Ag₃PO₄ is located lower than that of TiO₂, holes that are photogenerated in Ag₃PO₄ can transfer to TiO₂, and these energetic holes in the VB of TiO₂ are, in turn, able to initiate various oxidation reactions. Therefore, combining Ag₃PO₄ with TiO₂ to form heterostructures is regarded as an effective strategy for achieving efficient photocatalysts with high photocatalytic activities under visible-light irradiation.²²−²⁵ Moreover, Teng et al. found that the silver (Ag⁰) nanoparticles formed on the
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surface of Ag₃PO₄ can act as electron acceptors to enhance the charge separation and prevent the reductive decomposition of Ag₃PO₄. At the same time, the effect of the surface plasmon resonance of metallic silver can further increase the absorption of visible light. Nevertheless, despite its well-known photocatalytic activity under visible light, still very few research groups have attempted to employ Ag₃PO₄ as an antibacterial material by the photoinactivation process. Moreover, in practical application, it is relatively difficult to isolate and recover nanosized photocatalysts from a mixed suspension after a photocatalytic reaction to avoid secondary contamination. Magnetic photocatalysts represent an alternative for addressing this problem. These photocatalysts can be easily recovered by a magnet and can be recovered from a photocatalysis reaction system several times without any appreciable reduction in photocatalytic efficiency.

To the best of our knowledge, until now, no report has been focused on the preparation of magnetic Ag₃PO₄/TiO₂/Fe₃O₄ nanocomposite and the exploration of its antibacterial activity, even though the photocatalytic activities of Ag₃PO₄ and its hierarchical nanostructures are widely recognized and have triggered extensive research interest. The aim of the present work was to develop a magnetically recoverable photocatalyst with excellent photoinactivation properties toward bacteria under visible-light irradiation. Detailed investigations of the photocatalytic activity of Ag₃PO₄/TiO₂/Fe₃O₄ nanoparticles and their photoinactivation toward E. coli were carried out under visible-light irradiation. Compared to TiO₂ and Ag₃PO₄ the as-formed Ag₃PO₄/TiO₂/Fe₃O₄ nanocomposite was found to exhibit highly improved excellent performances in antimicrobial applications.

2. EXPERIMENTAL SECTION

2.1. Materials and Reagents. FeCl₃·6H₂O, FeCl₂·3H₂O, AgNO₃, Na₂HPO₄, Ti(SO₄)₂, and poly(ethylene glycol) (PEG-600) were purchased from Sigma-Aldrich Chemical Co. NH₃·H₂O, urea, tetraethyl titanate, and other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. and used without further purification. All aqueous solutions were prepared with deionized (DI) water (>18 MΩ). DI water was prepared by inserting a titanium tube into a water purification system (DI water treatment system; OMEI Nanotechnology Co. Ltd., Shanghai, China) and the DI water was then deionized by a deionization system (DI water purification system; Jaya Nanotechnology Co. Ltd., Shanghai, China).

2.2. Preparation of Ag₃PO₄/TiO₂/Fe₃O₄ Nanospheres. To prepare Fe₃O₄ nanoparticles, 5.4 g of FeCl₃·6H₂O, 4.5 g of FeCl₂·3H₂O and 11.4 g of NH₃·H₂O were added to 22.75 mL of DI water. Then, 0.55 mL of Ti(SO₄)₂ (0.5 M) was added to the mixture and the solution was sonicated for 2 h. Subsequently, 3.0 mL of Na₂HPO₄ solution (0.15 M) was added, and the solution was sonicated for 2 h. Finally, the suspension was annealed in Ar at 360°C for another 2 h.

The deposition of Ag₃PO₄ nanoparticles onto the TiO₂/Fe₃O₄ surface was carried out by the precipitation method. Briefly, 5.0 mg of TiO₂/Fe₃O₄ powder was dispersed in 20.0 mL of DI water; then 1.0 mL of Na₂HPO₄ (0.15 M) was added, and the solution was sonicated for 2 h. Subsequently, 3.0 mL of AgNO₃ solution (0.15 M) was dropped into this solution. The resulting nanocomposites were redispersed in 20.0 mL of DI water and then sealed in a Teflon-lined stainless steel autoclave for hydrothermal treatment at 160°C for another 2 h.

2.3. Photocatalytic Tests. To evaluate the photocatalytic activities of the photocatalysts, the performance of as-formed photocatalyst was evaluated in the degradation of azo dye acid orange 7 (AO7, C₈H₁₀N₂O₅S) in water. In a typical photocatalytic reaction, an amount of photocatalyst was added to a solution of AO7 (15 μM, 3.0 mL), and the mixture was kept in the dark for 30 min under continuous stirring to achieve the equilibrium adsorption of AO7 on the photocatalyst surface. Photocatalytic degradation was carried out by illuminating the suspension with visible light (ca. 0.8 cm²) using a 50-mW diode blue laser (λ = 405 nm). All photocatalytic experiments were carried out at room temperature under continuous stirring. The degradation of AO7 was monitored by UV–vis spectroscopy at different time intervals. To test the recyclability of the catalyst, the Ag₃PO₄/TiO₂/Fe₃O₄ nanoparticles were separated with an external magnet after AO7 had been completely decomposed and washed under flowing DI water three times before the next photocatalytic reaction.

2.4. Preparation of Photocatalyst-Containing Antibacterial Films. In general, suspensions of the as-prepared photocatalyst nanoparticles (0.3 mg) were dripped onto filter paper (Φ 15 mm) separately. After being allowed to dry in air, the filter papers were then placed onto the bottom of a Petri dish for antibacterial experiments. Control samples were prepared by using filter paper without loading any nanoparticles. After the antibacterial tests, the films were washed with phosphate-buffered saline (PBS, 10 mM, pH 7.0) to remove the entrapped bacterial bodies. The films were then dried and stored at room temperature.

2.5. Antibacterial Tests. Antibacterial activities were evaluated using Gram-negative DH5α Escherichia coli (E. coli). The bacterial suspensions employed for the test contained 10⁷ colony-forming units per milliliter (CFU mL⁻¹). Before the microbiological experiments, all glasswares was sterilized by autoclaving at 121°C for 30 min. For each antibacterial experiment, the prepared photocatalyst-containing antibacterial film was placed on the bottom of a Petri dish containing 10 mL of E. coli suspension. Then, the antibacterial experiment was initiated by irradiating the suspension with a commercial 300-W xenon lamp equipped with a UV cutoff filter (λ > 420 nm). The light source was located 20 cm from the reaction solution (50 mW cm⁻² illumination intensity). After irradiation, 1 mL of the treated bacterial suspension was withdrawn and diluted serially with sterilized Milli-Q water to adjust the bacterial concentration (aliquots were diluted to 10⁻⁵ times in the present study) to ensure that the growing bacterial colonies were being counted accurately and easily. To quantify the antimicrobial results, 100 μL of the treated suspension was spread on a lysogeny broth (LB) agar plate (LB liquid medium with 1.5% agar), and the colonies were counted to determine the survival bacterial numbers after incubation at 37°C for 24 h. The bactericidal efficiency of the photocatalyst-containing film was determined by comparing the corresponding colony counts with those obtained for control films (prepared in the dark without addition of nanoparticles), according to the equation:

\[
\text{bactericidal efficiency (\%)} = \frac{N_{\text{control}} - N_{\text{sample}}}{N_{\text{control}}} \times 100\% \tag{1}
\]

where \(N_{\text{control}}\) and \(N_{\text{sample}}\) correspond to the numbers of colonies counted in the control and sample agar plates, respectively.

2.6. Disk Diffusion Assays. E. coli suspension (10⁷ CFU/mL) was spread on the surface of LB plates. Sterilized Oxford cups (Φ 6 mm) were then placed on the plates and filled with 0.1 mg of photocatalyst. After exposure to visible light for 5 min, the plates were incubated at 37°C for 24 h. All tests were performed in triplicate, and the results were averaged.

2.7. Detection of OH Radicals. Terephthalic acid (TA) was used without further purification. An aqueous solution containing 0.01 M NaOH, 0.1 M KCl, 0.3 mM H₂O₂, and 3 mM TA was prepared, and then 0.3 mg of Ag₃PO₄ or Ag₃PO₄/TiO₂/Fe₃O₄ powder was suspended in 3.5 mL of this solution in a 1 cm × 1 cm quartz cuvette. The sample cell was placed on a magnetic stirrer and irradiated under a diode blue laser (λ = 405 nm) for 0–90 min. Fluorescence spectra of the supernatant liquid were measured with a fluorescence spectrophotometer (Hitachi F-7000).
2.8. Apparatus. The morphologies of the nanoparticles and bacteria were characterized by field-emission scanning electron microscopy (FE-SEM; Hitachi S-4800, Tokyo, Japan) and transmission electron microscopy (TEM; JEOL 2000). X-ray diffraction (XRD) patterns were acquired on an X’Pert X-ray diffraction spectrometer (Philips, Guildford, Surrey, U.K.) with a Cu Kr X-ray source. UV–vis absorption spectra were measured on a spectrophotometer (Perkin-Elmer, Lambda XLS+, Wellesley, MA). The size of the nanoparticles was analyzed on a Zetasizer Nano ZS90 instrument (Malvern Instruments, Malvern, U.K.). X-ray photoelectron spectroscopy (XPS) was performed on a Perkin-Elmer Physical Electronics 5600 spectrometer using Al Kα radiation at 13 kV as the excitation source. The takeoff angle of the emitted photoelectrons was 45°, with a resolution of 0.1 eV. Inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500) was used for the quantification of leached silver ions. Fluorescence emission spectra were recorded on a Hitachi F-7000 type fluorescence spectrometer.

3. RESULTS AND DISCUSSION

3.1. Structural Characterization of As-Prepared photocatalysts. The mean diameter of the Fe3O4 nanoparticles was ∼10 nm (Figure S1, Supporting Information). After a layer of TiO2 was wrapped around the Fe3O4 surface, the diameter of TiO2/Fe3O4 increased to 100 ± 30 nm (denoted as TiO2/Fe3O4, Figure S2, Supporting Information). Because of the magnetism of Fe3O4, some agglomerations of TiO2/Fe3O4 nanoparticles appeared. In Figure 1, the morphology of the as-prepared Ag3PO4/TiO2/Fe3O4 composites was investigated by field-emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). It can be seen that the diameter of the synthesized Ag3PO4/TiO2/Fe3O4 hierarchical nanoparticles was about 200 ± 50 nm after Ag3PO4 had been loaded. The as-formed Ag3PO4/TiO2/Fe3O4 nanoparticles were well-dispersed in water, and the suspended nanoparticles could be easily drawn to the side wall of the vial with a magnet (inset of Figure 1A), implying good magnet-controlled properties and recyclability. The decorated Ag3PO4 nanoparticles could also be distinguished on the hierarchical nanostructure by high-resolution TEM (HRTEM, Figure 1C) with sizes ranging from 5 to 10 nm. These Ag3PO4 nanoparticles were in intimate contact with the TiO2 support, and the lattice fringes of both Ag3PO4 and TiO2 can be clearly identified in Figure 1D. The lattice constant of about 0.65 nm is consistent with the body-centered-cubic (bcc) structure of Ag3PO4 and the lattice spacing of 0.35 nm is in agreement with the spacing of anatase TiO2 (101) lattice planes (ICDD-JCPDS database, 21-1272). Furthermore, the distribution of each element in Ag3PO4/TiO2/Fe3O4 was examined by high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and energy-dispersive X-ray spectroscopy (EDS) measurements. The results are shown in Figure S4 (Supporting Information). As manifested by the cross-sectional compositional line profile, Ag was found throughout the whole nanoparticle with very high intensity, whereas Fe and Ti were detected only near the center region, at a lower intensity.

Ag3PO4/TiO2/Fe3O4 nanoparticles exhibited a bcc structure. The diffraction peaks of Ag3PO4, TiO2/Fe3O4, and Ag3PO4/TiO2/Fe3O4 are presented in Figure 2A. The Ag3PO4 nanoparticles exhibited a bcc structure. The diffraction peaks

![Figure 1](image1.png)

**Figure 1.** (A) SEM and (B) TEM images of the as-synthesized Ag3PO4/TiO2/Fe3O4 nanospheres. (C) HRTEM images of Ag3PO4 particles on the Ag3PO4/TiO2/Fe3O4 nanospheres. (D) Spacing of TiO2 and Ag3PO4 lattice fringes. Inset in panel A: Photographs of Ag3PO4/TiO2/Fe3O4, which were easily dispersed in water (left) and drawn from the solution to the sidewall of the vial by an external magnet (right).

![Figure 2](image2.png)

**Figure 2.** (A) XRD patterns and (B) UV–vis absorption spectra of Ag3PO4, TiO2/Fe3O4, and Ag3PO4/TiO2/Fe3O4 nanoparticles.
The intensity of the Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ suspension was enhanced by light from a laser at 405 nm without using any sacrificial reagent. The effect of the concentration of Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ on the speed of photocatalytic degradation was investigated first by adding different quantities of Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ nanoparticles to AO7 aqueous solutions. As shown in Figure 3A, AO7 degradation occurred only after the addition of Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ nanoparticles, and the AO7 degradation rate increased with increasing photocatalyst concentration. For example, only ~45% of AO7 was decomposed after 2 min when the Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ suspended concentration was 0.1 mg/mL, but most of the AO7 could be decomposed in 2 min when the photocatalyst concentration reached 0.5 mg/mL. Compared to the results recorded for Ag$_3$PO$_4$ (Figure S8, Supporting Information), it is obvious that AO7 photodegraded more rapidly on Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ nanocomposite at the same nanoparticle concentration. Furthermore, it was also noticed that the Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ nanoparticles became dark after the photocatalytic reaction. The same color change was also observed on the Ag$_3$PO$_4$ nanoparticles. A similar phenomenon was also observed by other groups, who reported that this color change could originate from the formation of Ag$_0$ species through the reduction of Ag$_3$PO$_4$ by photoinduced electrons.

To further investigate the influence of the hierarchical structure on photocatalytic ability, the photocatalytic activity in the degradation of AO7 under visible light was compared using Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$, TiO$_2$, and Ag$_3$PO$_4$ nanoparticles as the photocatalysts. (TiO$_2$ and Ag$_3$PO$_4$ nanoparticles were prepared by a similar approach as described in the Experimental Details in the Supporting Information, and the morphologies are characterized in Figure S3 of the Supporting Information.) As displayed in Figure 3B, because of the weak absorption of pure TiO$_2$ in the visible-light region, TiO$_2$ nanoparticles showed only a very low photocatalytic activity for AO7 degradation under visible-light irradiation. On the other hand, because of the small band gap of Ag$_3$PO$_4$, the utilization of visible light was improved efficiently. As expected, the as-formed Ag$_3$PO$_4$ and Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ samples showed significantly enhanced photocatalytic activities in the degradation of AO7 under visible light. Moreover, the Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ composites exhibited a much higher photocatalytic activity than the Ag$_3$PO$_4$ nanoparticles.

The stability of a photocatalyst is an important parameter in practical applications. Herein, the activity and stability of Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ were evaluated by examining its recyclability in the photocatalytic degradation of AO7. The photocatalytic stabilities of the catalysts were tested in five successive AO7 degradation experiments using the same Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ sample (1.0 mg/mL). For comparison, the recyclabilities of TiO$_2$ and Ag$_3$PO$_4$ were tested under the same conditions. As plotted in Figure 4, after five photocatalytic experiments, the photodegradation ability of the Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ sample was almost identical to that of the fresh sample. The degraded AO7 decreased from 86% to 67% in five successive experimental runs when using Ag$_3$PO$_4$ as the photocatalyst, whereas the degraded AO7 decreased from 48% to 37% when TiO$_2$ was used as the photocatalyst. The amount of silver ions released from the photocatalysts was also investigated. Figure S9A (Supporting Information) shows a plot of the released silver ion concentration as a function of time. The quantity of silver ion released from the Ag$_3$PO$_4$ sample increased sharply in the next few minutes.
beginning, whereas the release of silver ions from Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ was relatively slow. For both photocatalysts, the silver ion concentration was noticed to decrease after 30 min, which can be attributed to the reduction of Ag$^+$ to Ag$^0$ on the photocatalyst surface by the photogenerated electrons.

### 3.3. Photocatalytic Antibacterial Characteristics

Concerning the prospective application of Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ under visible-light exposure, the photocatalytic antibacterial properties of the as-synthesized catalyst was investigated in the present work. *Escherichia coli* (*E. coli*), which is responsible for many infections in daily life, served as the model target microorganism. As illustrated in Scheme 1, the bactericidal efficiency against *E. coli* was studied for an antibacterial film, which was prepared by loading the photocatalyst onto filter paper. First, the effects of the presence of photocatalyst and visible light ($\lambda > 400$ nm) were explored. As shown in Figure S10 (Supporting Information), visible-light irradiation alone did not show any significant bactericidal effects on *E. coli* in the absence of photocatalyst. After 0.30 mg of Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ nanoparticles had been loaded, ~42% of *E. coli* cells were inactivated after 50 min even in the dark, which strongly indicates that Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ nanoparticles are particularly cytotoxic to *E. coli*. In addition, a marked enhancement of bactericidal efficiency was observed by combining the photocatalyst with visible-light irradiation. In this case, the *E. coli* cells were completely inactivated. Furthermore, inhibitory concentration tests to quantitatively compare the bactericidal efficiency were carried out. As plotted in Figure S11 (Supporting Information), the presence of 0.15 mg of Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ nanoparticles already showed an obvious suppression of bacterial growth with about 80% inactivation of *E. coli* (10$^7$ CFU/mL) under visible-light irradiation. The complete suppression of bacterial growth was observed when the Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ loading content was increased to 0.30 mg.

To evaluate the antibacterial activity of the Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ hierarchical nanostructure, the bactericidal efficiency of Ag$_3$PO$_4$/TiO$_2$/Fe$_3$O$_4$ nanocomposite was compared to the activities of the other photocatalysts. As shown in Figure 5A, a negligible antibacterial activity was observed in the absence of photocatalyst (curve a). Because of the lower photocatalytic activity of TiO$_2$ under visible light, only 56% inactivation of *E. coli* was found for the TiO$_2$ sample even after 50 min of irradiation using visible light (curve b). In contrast, marked enhancements in bactericidal efficiency were observed on the Ag$_3$PO$_4$ (curve c)
and Ag₃PO₄/TiO₂/Fe₃O₄- (curve d) based antibacterial films upon visible-light illumination. In particular, ∼99.8% of the E. coli was inactivated on the Ag₃PO₄/TiO₂/Fe₃O₄-loaded film only upon irradiation for 5 min (curve d). Additionally, by comparing the bactericidal results on Ag₃PO₄/TiO₂/Fe₃O₄-based antibacterial films in the presence (curve d) and absence (curve e) of visible-light irradiation, it is evident that, apart from the effect of the silver ions, a large part of the bactericidal effects is related to the photocatalytic process.39

Stability and reusability are also two major challenges in employing photocatalytic materials for antimicrobial applications. To evaluate the antimicrobial stabilities of the as-formed nanocomposites, we further studied the inactivation of Ag₃PO₄-, TiO₂-, and Ag₃PO₄/TiO₂/Fe₃O₄-based antibacterial films for several bactericidal cycles. In this study, the as-prepared antibacterial films were reused in five successive bactericidal experiments. The antibacterial films were cleaned by washing with DI water three times before the next photoinactivation of bacteria. As shown in Figure 5B, although some antibacterial activity losses were also observed for the Ag₃PO₄/TiO₂/Fe₃O₄ film, the bactericidal efficiency from Ag₃PO₄ films exhibited more significant decreases under the same conditions. Moreover, tests of storage stability revealed that the Ag₃PO₄/TiO₂/Fe₃O₄ nanoparticles kept ∼97% of their bactericidal activity against E. coli after being stored at room temperature for 30 days. Figure S9B (Supporting Information) shows a plot of the silver remaining (measured by centrifuging and dissolving the remaining photocatalyst in HNO₃) after the reuse of the same Ag₃PO₄/TiO₂/Fe₃O₄ and Ag₃PO₄ samples for the photoinactivation of E. coli. Compared to Ag₃PO₄, the silver concentrations in the Ag₃PO₄/TiO₂/Fe₃O₄ nanocomposite decreased much more slowly. This tendency is coincident with the change in bactericidal efficiency in Figure 5B. The results firmly demonstrate that Ag₃PO₄/TiO₂/Fe₃O₄-based antibacterial films have satisfied reusability and long-term stability at room temperature.

Qualitative antimicrobial disk diffusion tests were carried out by the Oxford cup method. The E. coli (10⁷ CFU/mL) was spread on the surface of an LB plate. The as-prepared photocatalyst (0.1 mg) was then filled into an Oxford cup on the LB plate. After exposure to visible light for 5 min, the plate was incubated at 37 °C for 24 h in the dark. As shown in Figure 6A, the Ag₃PO₄ and Ag₃PO₄/TiO₂/Fe₃O₄ nanoparticles exhibited clearly visible inhibition zones for E. coli, with average diameters of 19 and 24 mm, respectively. The differences in diameter suggest that the Ag₃PO₄/TiO₂/Fe₃O₄ nanocomposite had better antimicrobial effects against E. coli under visible-light irradiation. The weak antibacterial activities observed for the TiO₂ nanoparticles confirmed that visible light has an insufficient influence on the wide-band-gap photocatalyst. To further understand how the Ag₃PO₄/TiO₂/Fe₃O₄ nanocomposite can access and kill E. coli, FE-SEM was applied to characterize the...
bacterial cells before and after the photoinactivation process. As shown in Figure 6B, the intact \( E. \ coli \) cells presented a well-defined cell wall. After the damage of \( E. \ coli \) on \( \text{Ag}_3\text{PO}_4/\text{TiO}_2/\text{Fe}_3\text{O}_4 \) nanocomposite by visible-light irradiation, clear destruction was observed, with the outer membrane collapsed and presented as circles (Figure 6C). As a widely used antibacterial material, silver ions can interact with the main components of the bacterial cell, including the peptidoglycan cell wall and the plasma membrane, cytoplasmic DNA, and bacterial proteins, and can also induce collapse of the bacterial membranes during performance of the antibacterial function. In the current study, the \( \text{Ag}_3\text{PO}_4/\text{TiO}_2/\text{Fe}_3\text{O}_4 \) nanoparticles were found to anchor closely to the outer walls of the \( E. \ coli \) cells. These results are consistent with the phenomena observed by Zeng et al., who reported that this close-contact configuration could further enhance the photocatalytic activity of the semiconducting composite. A magnified image of \( E. \ coli \) is displayed in Figure 6D. Obviously, the outer membrane of the \( E. \ coli \) cell is destroyed as well as the ends of the cell after the photoinactivation process.

### 3.4. Mechanism for the Highly Improved Photocatalytic Antibacterial Activity of \( \text{Ag}_3\text{PO}_4/\text{TiO}_2/\text{Fe}_3\text{O}_4 \)

The surface components and composition of \( \text{Ag}_3\text{PO}_4/\text{TiO}_2/\text{Fe}_3\text{O}_4 \) nanoparticles before and after bactericidal experiments were investigated by XPS analysis. The XPS results in Figure 7 show the main difference in the surface composition of the two different nanoparticles in the P 2p and Ag 3d regions. The P 2p peak of \( \text{Ag}_3\text{PO}_4 \) (Figure 7A) was found to decrease significantly after being reused in two and five successive photocatalytic experiments, which can be attributed to the dissolution of \( \text{Ag}_3\text{PO}_4 \). The P 2p peak of \( \text{Ag}_3\text{PO}_4/\text{TiO}_2/\text{Fe}_3\text{O}_4 \) (Figure 7B) exhibited a slight decrease. For the Ag 3d signal, the two broad peaks located at 367.5 and 373.6 eV can be assigned to the doublet Ag 3d\( _{5/2} \) and Ag 3d\( _{3/2} \), respectively, of Ag\( ^+ \) ions. After the photocatalytic reaction, the Ag 3d\( _{5/2} \) and Ag 3d\( _{3/2} \) peaks of \( \text{Ag}_3\text{PO}_4 \) showed an obvious decrease and shift to 368.2 and 374.2 eV, respectively. The observed positive shifts can be assigned to the formation of metallic Ag\( ^0 \). Similar results have been reported by other researchers.

**Figure 7.** Details of the (A,B) P 2p and (C,D) Ag 3d peaks of the prepared (A,C) \( \text{Ag}_3\text{PO}_4 \) and (B,D) \( \text{Ag}_3\text{PO}_4/\text{TiO}_2/\text{Fe}_3\text{O}_4 \) nanoparticles after use in the photocatalytic reaction for 0, 2, and 5 cycles.
catalysis reaction and Ag⁺ ions in solution can be reduced to Ag⁰ on the surface of the photocatalyst by the photogenerated electrons, the Ag/P atomic concentration ratio increased with the number of cycles. Compared to Ag₃PO₄/TiO₂/Fe₃O₄ nanoparticles, the Ag/P atomic concentration ratio of Ag₃PO₄ nanoparticles increased sharply. Obviously, for Ag₃PO₄/TiO₂/Fe₃O₄ nanocomposite, the dissolution of Ag₃PO₄ was slower than that of Ag₃PO₄ itself, which indicates that the Ag₃PO₄/TiO₂/Fe₃O₄ nanocomposite was more stable in photocatalytic reaction than the Ag₃PO₄. These results are consistent with the ICP-MS results in Figure S9A (Supporting Information).

To gain more insight into the possible mechanism of the photocatalytic degradation of organic dyes and photoinactivation of bacteria, we performed some additional experiments. It is often perceived that the active oxygen-containing radicals generated in the irradiated system relate to the photoinduced antibacterial activity. To test for *OH radicals, we employed a fluorescence approach based on a terephthalic acid (TA) reaction, which is frequently used in photocatalysis investigations. It is based on the reaction of *OH with TA to form 2-hydroxyterephthalic acid (TAOH) that exhibits a characteristic blue fluorescence at 426 nm. Figure 8A shows the fluorescence response observed for the supernatant solution of Ag₃PO₄ and Ag₃PO₄/TiO₂/Fe₃O₄ suspensions containing 3 mM TA irradiated for identical times (90 min). Because the observed fluorescence spectra were identical to that of TAOH, it is concluded that TAOH was generated from TA by the reaction with *OH, where *OH was formed during photocatalysis. The fluorescence intensity was plotted (Figure 8B) as a function of the duration of irradiation. Additionally, as exhibited in Figure 8C, a strong blue fluorescence was observed for the solution where Ag₃PO₄/TiO₂/Fe₃O₄ nanoparticles were added, and less fluorescence was seen for the Ag₃PO₄ nanoparticles. These results suggest that the Ag₃PO₄/TiO₂/Fe₃O₄ hierarchical structure is helpful in producing *OH radicals and favorable for enhanced photocatalytic activity. As mentioned earlier, the antimicrobial activity could not be fully attributed to the dissolved amount of silver ions. In the current work, the photoinduced active oxygen-containing radicals might also impart additional antimicrobial activity.

Herein, the highly enhanced photodegradation and antimicrobial activities achieved on Ag₃PO₄/TiO₂/Fe₃O₄ can be assigned to its hierarchical structure. As illustrated in Scheme 2, electron–hole pairs were created within Ag₃PO₄ under the irradiation of visible light, and then the photogenerated holes in Ag₃PO₄ were transferred to the valence band (VB) of TiO₂ and further reacted with the absorbed H₂O molecules to form active oxidants such as hydroxyl radicals (*OH). Meanwhile, the photogenerated electrons in the conduction band (CB) migrated to the surface of Ag₃PO₄ and further reacted with absorbed oxygen to form oxidants such as superoxide ions (O₂⁻). During the photocatalytic process, Ag⁰ nanoparticles were produced by the partial reduction of Ag₃PO₄ by the photogenerated electrons. The resultant Ag metals could trap the photo-
generated electrons and thus inhibit the further decomposition of Ag3PO4. Therefore, the formed Ag/Ag3PO4/TiO2 interfaces can effectively promote charge separation and enhance the photocatalytic activity of the catalyst. In particular, because the as-prepared Ag3PO4/TiO2/Fe3O4 nanocomposite exhibits outstanding photocatalytic activity under visible light, the photogenerated reactive oxidants (OH and O2−) oxidize organic compounds/cells adsorbed on the Ag3PO4/TiO2/Fe3O4 surface and result in the death of the microorganisms. Therefore, the observed breakdown of cell membranes can be attributed to the cytotoxicity of silver ions eluted from the compounds and the formation of various reactive oxygen species on photocatalyst/water interfaces under visible-light irradiation.2,47

4. CONCLUSIONS

In summary, we have demonstrated in this study that the magnetic Ag3PO4/TiO2/Fe3O4 nanocomposite is an effective visible-light photocatalyst. The as-prepared Ag3PO4/TiO2/Fe3O4 nanoparticles can be well-dispersed in water and recovered by an external magnet. Owing to the low band gaps of Ag3PO4 and the formed Ag/Ag3PO4/TiO2 interface during the photocatalytic reaction, the Ag3PO4/TiO2/Fe3O4 hierarchical structure exhibited effective charge separation and excellent photocatalytic activity. The photogenerated oxidants (OH and O2−) formed on Ag3PO4/TiO2/Fe3O4 by visible-light illumination lead to a strong bactericidal effects toward E. coli.

■ ASSOCIATED CONTENT

* Supporting Information
Some experimental details, TEM and SEM images, XRD measurements, device used for the photocatalytic reaction, influence of catalyst concentration on the photocatalytic degradation, Ag+ concentration detected by ICP-MS, and effect of visible-light irradiation on the antibacterial activities. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
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