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ARTICLE

Antimicrobial effects of silver deposited on nanolayered manganese oxide

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Silver deposited on layered manganese oxide was synthesized by a simple method, and characterized by scanning electron microscopy, transmission electron microscopy, X-ray photoelectron spectroscopy, X-ray diffraction spectrometry, atomic absorption spectroscopy, and energy-dispersive X-ray mapping. In the next step, antimicrobial effects of the compound for *Escherichia coli* and *Staphylococcus aureus* were considered.

Introduction

The inhibitory and bactericidal effects by Ag and Ag compounds have been known for many years.¹ Many groups reported antimicrobial effects of Ag-containing dressings,² textiles,³ films,⁴ composite coatings,⁵ and dendrimers⁶ on as many as 12 species of bacteria including *Escherichia coli*. The mechanism of the effect of Ag on these bacteria is not known, but different groups proposed that Ag(I) has effect on uncouple the respiratory chain from oxidative phosphorylation,⁷ collapse the proton-motive force across the cytoplasmic membrane,⁸ interact with thiol groups of membrane-bound enzymes and proteins,⁹ and interaction with nucleic acids.^{10a} Ag in very low concentration is not toxic to human cells.^{10b} Thus, it can be considered an environmentally friendly antimicrobial compound. Ag nanoparticles are new forms of bactericidal Ag-based compounds, and there are many reports to show antimicrobial activity of Ag nanoparticles.¹¹⁻¹⁶ The mechanism of antimicrobial activity of Ag nanoparticles is not known, but two hypothesizes are presented. Some groups suggested that Ag could release Ag(I) from its surface, and the ion can interact with the cellular targets,¹¹⁻¹³ In this case, Ag nanoparticles have high surface, and can release more Ag(I). On the other hand, other groups reported evidences that the antibacterial effect of Ag nanoparticles may arise from the direct effect of these nanoparticles.¹⁴⁻¹⁶

It is interesting that Nature uses Mn oxides to control heavy metals and other trace elements in soils and aquatic sediments.^{17,18} Layered Mn oxides are a kind of ubiquitous hydrous-layered Mn oxide in geological environments.¹⁹ The compound could be used to remove heavy metal ions (Pb(II), Cd(II), As(III), Cr(III) and etc.) from water systems.²⁰ Thus, we combine antibacterial activity of Ag, and the activity of layered Mn oxide to remove heavy metal ions to obtain one compound.

Experimental

Material and methods

All reagents and solvents were purchased from Merck or Sigma-Aldrich, and were used without further purification.

Synthesis

Synthesis of nanolayered Mn oxide

It was synthesized by a previously reported method.^{21,22} Solution 1: $\text{Mn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ (2.77 mmol, 692.0 mg) was dissolved in the smallest possible amount of water. Solution 2: to a solution of KMnO_4 (2.00 mmol, 316.0 mg) in 35 mL water contains KOH (40.0, 20.0, 10.0 gr). Addition of solution 1 to solution 2 under vigorous stirring resulted in a dark brown precipitate. The obtained suspension was filtered and washed using distilled water (1.0 L) before being allowed to dry for 12 h at 60 °C in an oven.

Synthesis of silver deposited on nanolayered Mn oxide

It was synthesized by a previously reported method.^{21,22} 100.0 mg of the layered Mn oxide was added to a solution contains AgNO_3 (10 mL, 60 mg), and the mixture was stirred for 2 h. The solid was washed once with water, and dried at 300 °C.

Characterization

SEM was carried out with LEO 1430VP. The X-ray powder patterns were recorded with a Bruker D8 ADVANCE diffractometer (CuK_α radiation). Mn atomic absorption spectroscopy (AAS) was performed on an Atomic Absorption Spectrometer Varian Spectr AA 110. Prior to analysis, each oxide (2.0 mg of oxide) was added to 1 mL of concentrated nitric acid and H_2O_2 , and left at room temperature for at least 1 h to ensure that the oxide was completely dissolved. The solutions were then diluted and analyzed by AAS.

Cell Preparation

The bactericidal activities were considered against *Escherichia coli* (ATCC 25922) (Gram-negative) and *Staphylococcus aureus* (ATCC 25923) (Gram-positive bacteria). All the samples and glassware

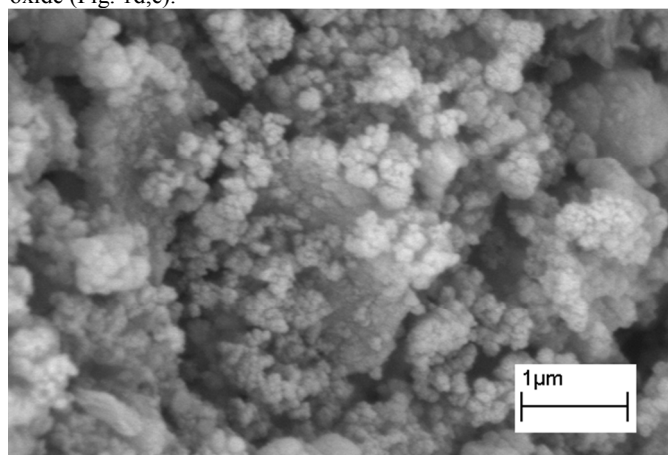
were sterilized by autoclaving at 120 °C for 10 min before the experiments. In our procedure, both of bacteria were grown in LB Luria Bertani medium at 37 °C, and harvested in the mid-exponential growth phase. Cultures were centrifuged at 6000 rpm for 10 min to pellet cells, and cells were washed three times with isotonic saline solution to remove residual macromolecules, and other growth medium constituents. The pellets were then resuspended in isotonic saline solution. Bacterial cell suspensions were diluted to obtain cell samples containing 10^6 to 10^7 CFU/mL.^{23,24}

Cell Viability Test

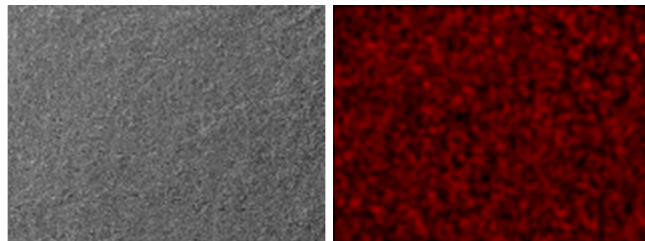
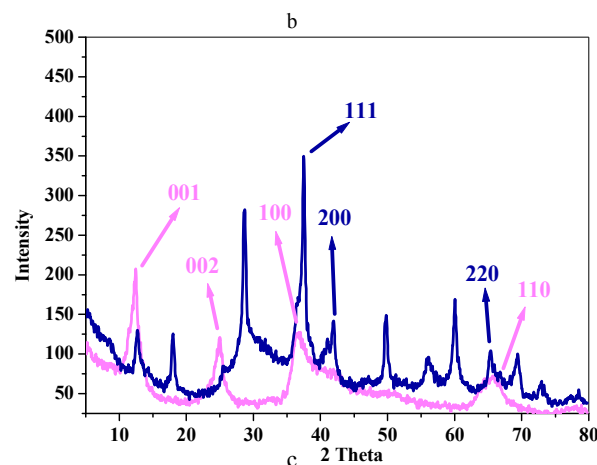
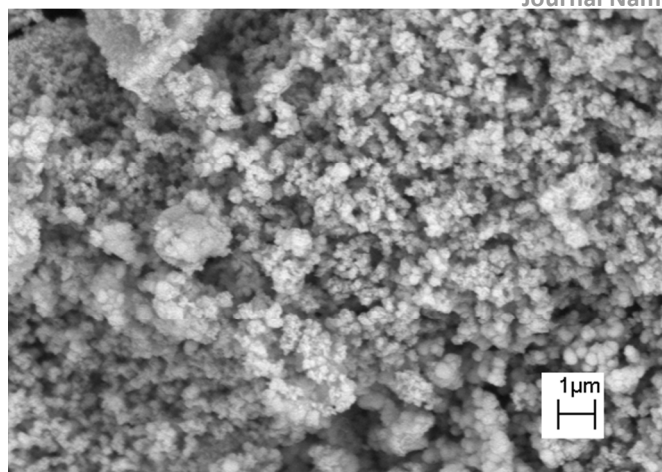
Escherichia coli and *Staphylococcus aureus* cells were incubated with nanolayered Mn oxide, and Ag deposited on nanolayered Mn oxide dispersions in isotonic saline solutions at 37 °C under 250 rpm shaking speed for reported time. The survival of bacterial of *Escherichia coli* and *Staphylococcus aureus* cells were tested by colony counting method. Briefly, series of 10-fold cell dilutions (100 μ L each) were spread onto Luria Bertani plates, and left to grow overnight at 37 °C. Colonies were counted and compared with those on control plates to calculate changes in the cell growth inhibition. Isotonic saline solution without Mn oxide-based compounds was used as control. All treatments were prepared in duplicate, and repeated at least on three separate occasions.^{23,24}

Results and discussion

SEM images for nanolayered Mn oxide (Fig. 1a), and Ag deposited on nanolayered Mn oxide (Fig. 1b) show nanoparticles in the range of 20-100 nm (diameter). XRD patterns for nanolayered Mn oxide and Ag deposited on nanolayered Mn oxide are shown in Fig. 1c. The results show patterns for (001), (002), (100) and (110) for nanolayered Mn oxides. For Ag deposited on nanolayered Mn oxide in addition to layered Mn oxide, the three strong diffraction peaks located at 38, 44, and 64° can be indexed as the (111), (200), and (220) planes of metallic Ag (Fig. 1c).²⁵ EDX-mapping shows good dispersion of Ag on the surface of Ag deposited on nanolayered Mn oxide (Fig. 1d,e).



a



d

e

Fig. 1 SEM images for nanolayered Mn oxide (a), and Ag deposited on nanolayered Mn oxide (b). XRD patterns nanolayered Mn oxide (pink) and Ag deposited on nanolayered Mn oxide (royal) (c). SEM (d) and SEM-EDX mapping (e) for Ag dispersion on the surface of Ag deposited on nanolayered Mn oxide.

TEM images show that nanolayered Mn oxide (Fig. 2a) and Ag deposited on nanolayered Mn oxide (Fig. 2b) contain nanolayers of Mn oxide. Ag nanoparticles can be observed as very small and dark particles in TEM image.

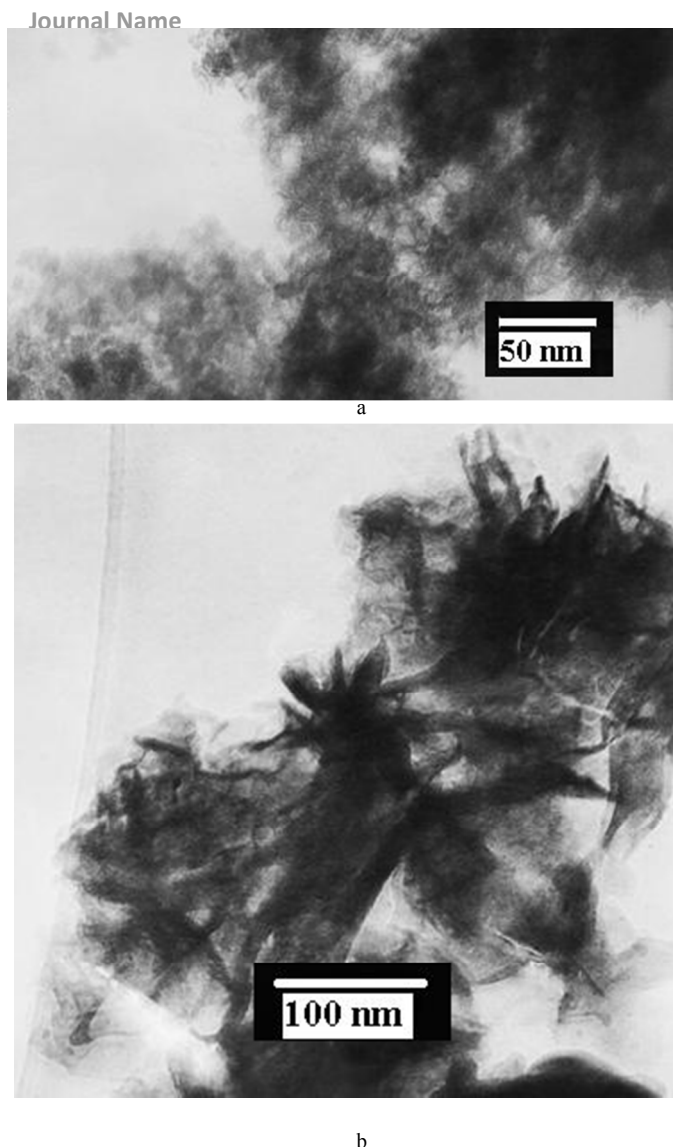


Fig. 2 TEM images for nanolayered Mn oxide (a), and Ag deposited on nanolayered Mn oxide (b).

XPS spectra show Ag, Mn and O on the surface of oxide at 368, 642, and 532 eV, respectively (Fig. 3). The oxidation states for Mn ions based on XPS spectrum are III and IV that are usual for oxidation state of Mn ions in layered Mn ions.²⁵

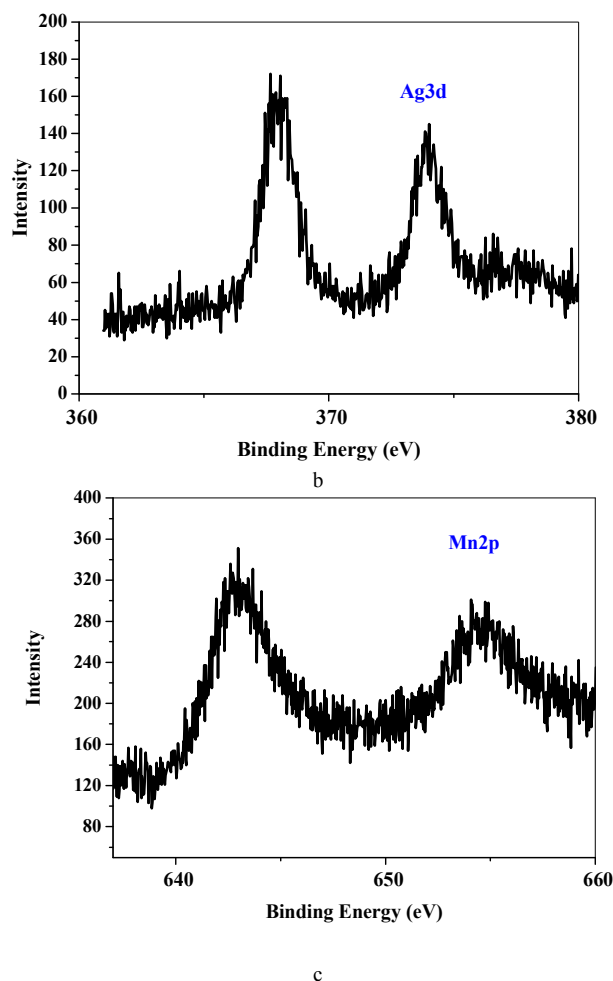
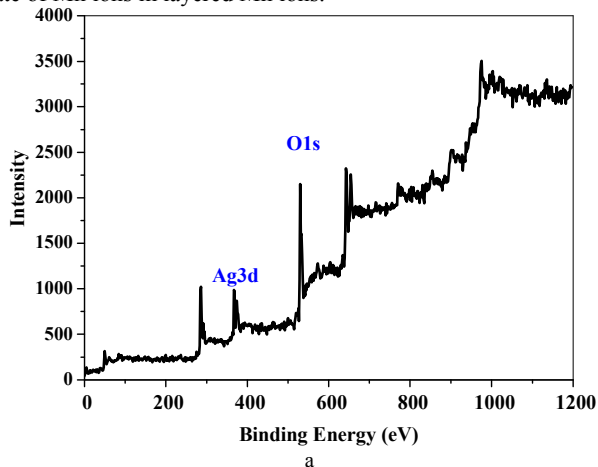


Fig. 3 XPS spectra for Ag deposited on nanolayered Mn oxide (a), and in the area of Ag (b) and Mn (c).

Next, we studied the antimicrobial effects of nanolayered Mn oxide, and Ag deposited on nanolayered Mn oxide. The *Escherichia coli* and *Staphylococcus aureus* were used as model bacteria to test antibacterial activities of the compounds. *Escherichia coli* and *Staphylococcus aureus* cells (10^6 CFU/mL) were incubated with the same concentration (100, 250, 500 and 750 $\mu\text{g/mL}$) of the compounds in isotonic saline solution for 2, 4, and 6 hours, respectively. The survival of bacterial cells was determined by the colony counting method described in the antimicrobial test section. The isotonic saline solution without nanolayered Mn oxide or Ag deposited on nanolayered Mn oxide materials was used as a control. The shaking speed 250 rpm was used in all antibacterial assays. As shown in Fig. 4, Ag deposited on nanolayered Mn oxide shows good antibacterial activities on both *Escherichia coli* and *Staphylococcus aureus*. Although some particles of nanolayered Mn oxide or Ag deposited on nanolayered Mn oxide particles precipitate when the dispersions stand still for 2, 4 and 6 hours under the shaking condition, the particles are well suspended in the saline solution interacting with cells in all assays.

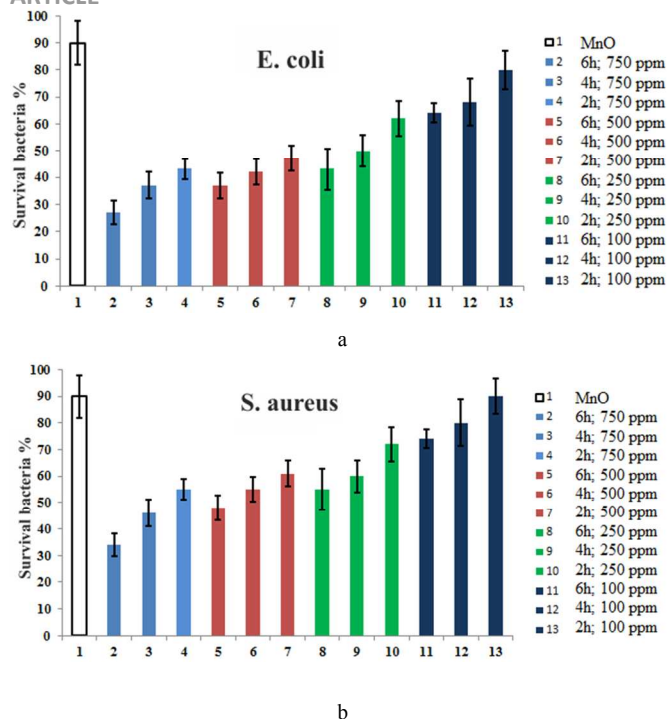


Fig. 4 Viable counts of (a) *Escherichia coli* and (b) *Staphylococcus aureus* bacteria after interaction by nanolayered Mn oxide (MnO), and Ag deposited on nanolayered Mn oxide.

Conclusions

We synthesized nanolayered Mn oxide, and Ag deposited on nanolayered Mn oxide. The compounds characterized by scanning electron microscopy, transmission electron microscopy, X-ray photoelectron spectroscopy, X-ray diffraction spectrometry, atomic absorption spectroscopy, and energy-dispersive X-ray mapping. The Ag deposited on nanolayered Mn oxide shows antibacterial activities toward *Escherichia coli* and *Staphylococcus aureus*. As layered Mn oxides are used to remove heavy metal ions (Pb(II), Cd(II), As(III), Cr(III) and etc.) from water systems, we according to deposit of Ag on nanolayered Mn oxide, combine both removing heavy-metal ions activity and antibacterial effect in one compound. The strategy is promising in water treatment.

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Notes and references

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