

The chitosan encapsulation strategy inhibits the dissolution of MoS₂/ZnO nanoparticles in water disinfection

Yutao Xie^{1,2,3}✉, Yunpeng Yu^{1,2,3}, and Feng Gao¹

¹Hefei National Research Center for Physical Sciences at the Microscale, University of Science and Technology of China, Hefei 230026, China;

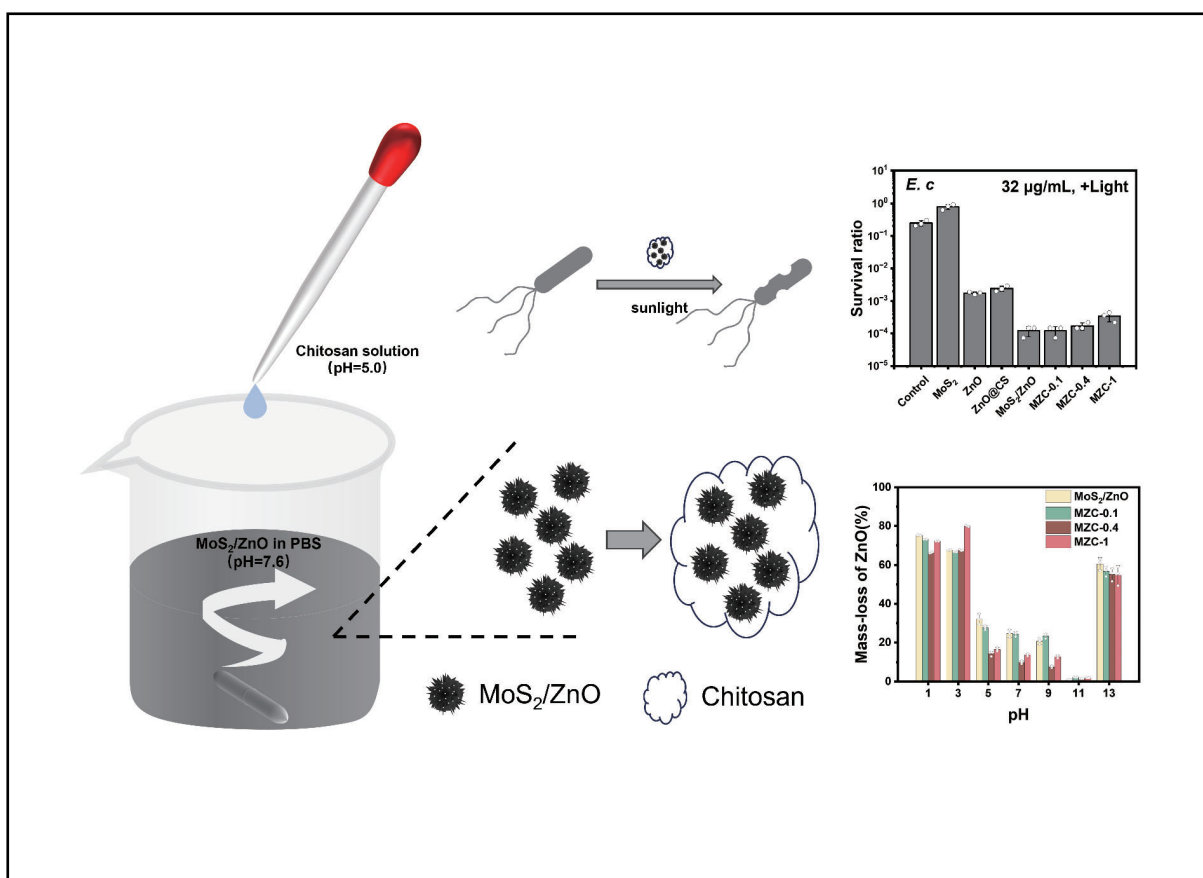
²CAS Key Laboratory of Soft Matter Chemistry, University of Science and Technology of China, Hefei 230026, China;

³School of Chemistry and Materials Science, University of Science and Technology of China, Hefei 230026, China

✉Correspondence: Yutao Xie, E-mail: ytxie@mail.ustc.edu.cn

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Graphical abstract



Preparation of a MoS₂/ZnO water disinfectant via a chitosan encapsulation strategy.

Public summary

- MoS₂/ZnO@CS composites were prepared via the precipitation method to encapsulate chitosan.
- The encapsulation of chitosan was found to inhibit the dissolution of ZnO in water.
- MoS₂/ZnO@CS composites have been demonstrated to exhibit high production of reactive oxygen species (ROS) and broad-spectrum antibacterial activity.

The chitosan encapsulation strategy inhibits the dissolution of MoS₂/ZnO nanoparticles in water disinfection

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²CAS Key Laboratory of Soft Matter Chemistry, University of Science and Technology of China, Hefei 230026, China;

³School of Chemistry and Materials Science, University of Science and Technology of China, Hefei 230026, China

 Correspondence: Yutao Xie, E-mail: ytxie@mail.ustc.edu.cn

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Supporting Information

Abstract: The combination of solar disinfection and photocatalysis technology presents a viable solution for eliminating harmful pathogenic microorganisms from water. However, some photocatalysts (e.g., zinc oxide-based composites) are susceptible to pH-dependent dissolution in water, which can result in the loss of photocatalysts and additional environmental pollution. To obtain zinc oxide-based composites with low dissolution and high antibacterial efficiency for photocatalytic water disinfection, we prepared MoS₂/ZnO@CS composites via a precipitation method to encapsulate chitosan (CS) around MoS₂/ZnO. The amino groups in the CS molecules act as storerooms for hydrogen ions, which inhibits the dissolution of zinc oxide. In addition, the MoS₂/ZnO@CS composites exhibit high production of reactive oxygen species (ROS) and broad-spectrum antibacterial activity under simulated solar irradiation (0.1 W·cm⁻²). This makes it an excellent antibacterial agent for solar disinfection in water treatment.

Keywords: ZnO; MoS₂; chitosan; antibacterial; water disinfection

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1 Introduction

In rural areas of developing countries, water sources are often contaminated and not disinfected for direct consumption due to poor infrastructure and sanitation^[1–3]. This poses a significant threat to the health of the local population, as undisinfected water may contain high levels of harmful microorganisms such as bacteria and viruses^[4]. Traditional disinfection methods, including chlorination^[5], ozone oxidation^[6], and ultraviolet (UV) irradiation^[7], can be used for water disinfection. However, these disinfection methods have several disadvantages, including high energy consumption, high technological costs, and the potential for toxic disinfection byproducts. In developing countries, the construction and maintenance costs of centralized water supply systems can be unaffordable due to the sparse populations in rural areas. Alternatively, solar disinfection (SODIS) is a method of purifying water by exposing it to sunlight^[8,9]. Studies have shown that UV light from sunlight interacts directly with nucleic acids or other biomolecules to cause inactivation^[10]. However, SODIS is time-consuming and requires 6–48 h of exposure^[8]. Therefore, there is a need to improve antibacterial efficiency by utilizing the photonic energy of sunlight more efficiently.

Compared with other disinfection methods, heterogeneous photocatalytic disinfection is a promising advanced oxidation process (AOP)^[11,12], with great potential to significantly reduce microbial contamination in the environment. It utilizes sunlight to generate reactive oxygen species (ROS), which act as environmentally friendly biocides^[13,14]. Currently, inorgan-

ic nanomaterials that have received research attention in the field of photocatalytic antimicrobials include zinc oxide (ZnO)^[15], titanium dioxide (TiO₂)^[16], copper sulfide (CuS)^[17], bismuth sulfide (Bi₂S₃)^[18], molybdenum sulfide (MoS₂)^[19] and graphene carbon nitride (g-C₃N₄)^[20]. Among these materials, ZnO and ZnO-based composites such as TiO₂/ZnO^[21] and MoS₂/ZnO^[22] stand out because of their low cost, high redox potential, and great biocompatibility. However, most studies have focused on the antibacterial efficacy of ZnO while neglecting the stability of ZnO in water^[23–26]. When using ZnO material for water disinfection, it is crucial to consider its potential to dissolve under aqueous conditions and produce Zn²⁺. This dissolution increases under acidic conditions^[27]. This potential dissolution not only decreases the catalytic performance but also increases the Zn²⁺ concentration in water. Studies have shown that ZnO exhibits biotoxicity to mammalian cells via a nanoparticle solubilization mechanism^[28,29], which is undesirable. Hirano et al.^[30] utilized a flame-assisted aerosol process to embed multiple ZnO nanoparticles in a TiO₂ shell layer, resulting in a multicore/shell structure. This structure results in increased acid resistance due to the presence of acid-resistant nanomaterials in the gaps between the multicore nanoparticles. We were motivated by the potential to use a polymer material that is more biocompatible as a protective layer. Chitosan (CS) is of interest because of its pH-dependent solubility^[31], which is similar to that of ZnO. Although antibacterial polymer–ZnO composites have been widely studied, the focus has focused primarily on the antibacterial properties that ZnO nanoparticles confer to polymer

matrices. However, to the best of our knowledge, no studies have focused on the ability of polymers to inhibit the dissolution of ZnO.

In this study, a MoS₂/ZnO@CS composite was prepared. The heterojunction formed by MoS₂ and ZnO resulted in high photocatalytic efficiency and antibacterial activity. CS was used to encapsulate MoS₂/ZnO through a precipitation method based on pH changes. The CS molecules, with many amino groups^[31], act as H⁺ storerooms, inhibiting the dissolution of MoS₂/ZnO, which protects the ZnO and effectively lowers the concentration of Zn²⁺ in the water. When the mass ratio of MoS₂/ZnO to CS was adjusted to 10 : 4, only 9.93% of the ZnO (32 µg·mL⁻¹) was dissolved in an aqueous solution (pH = 7.0), whereas the mass loss of ZnO in the bare MoS₂/ZnO reached 24.64%. Under identical conditions, we were able to achieve a sterilization ratio greater than 99.9% for four model bacteria under simulated sunlight (0.1 W·cm⁻²) for only 10 min. This work is expected to lead to the development of a polymer-inorganic antibacterial composite that exhibits low dissolution and ionic release, making it suitable for water disinfection.

2 Materials and methods

2.1 Materials

Zinc acetate dihydrate, ethanol, ethanolamine, chitosan, potassium dihydrogen phosphate, sodium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, nitric acid, ascorbic acid and hydrazine hydrate were purchased from Sino-pharm Chemical Reagent (Shanghai, China). Ammonium tetrathiomolybdate and zinc oxide nanoparticles (30 nm) were purchased from Aladdin (Shanghai, China). Tryptone soy broth (TSB) medium and bacterial agar powder were purchased from Haibo Biotechnology (Qingdao, China). A bacterial live/dead staining kit was purchased from Thermo Fisher Scientific (Shanghai, China). Phosphate buffer solution (PBS, 10×) and 2',7'-dichlorodihydrofluorescein (DCFH) were purchased from Beyotime Biotechnology (Shanghai, China). The strains used in this work were purchased from the American Type Culture Collection (ATCC) (Virginia, USA). Millipore water was used throughout the experiments. All reagents were used as received without any additional purification unless otherwise specified.

2.2 Preparation of MoS₂ and MoS₂/ZnO

First, 5.00 mg of (NH₄)₂MoS₄ was dissolved in 20.00 mL of ultrapure water, followed by the addition of 0.50 mL of N₂H₄·4H₂O. The resulting mixture was transferred to a hydrothermal kettle and reacted at 200 °C for 24 h. The precipitate was collected by centrifugation (10000 × g, 20 min) and washed three times with ultrapure water under the same centrifugation conditions. The prepared MoS₂ was stored as an aqueous dispersion.

The aqueous dispersion containing 3.25 mg of MoS₂ was centrifuged (10000 × g, 20 min) to remove the water. A total of 4.40 mg of zinc acetate dihydrate was weighed and dissolved in 20.00 mL of ethanol. The mixture was poured into MoS₂, sonicated for 10 min, and heated in a water bath with stirring at 600 r·min⁻¹ at 50 °C for 1 h. Afterward, ethanolam-

ine (0.02 mL) was added to the mixture, and the heating process was continued with stirring for a duration of 12 h. The resulting precipitate was collected through centrifugation (10000 × g, 20 min) and subsequently washed three times with ultrapure water under identical centrifugation conditions. Finally, the obtained MoS₂/ZnO composite was stored as an aqueous dispersion.

2.3 Preparation of MoS₂/ZnO@CS and ZnO@CS

MoS₂/ZnO@CS were prepared via a precipitation method. First, 27.22 g of potassium dihydrogen phosphate was dissolved in 1000.00 mL of water, from which 50.00 mL was added to 84.80 mL of 0.10 M NaOH solution, and then water was added to a total volume of 200.00 mL to obtain a phosphate buffer solution (pH=7.6). Next, 200.00 mg of CS was dissolved in 0.10 M HCl, the pH was adjusted to approximately 5.0 with NaOH, and water was added to a total volume of 100.00 mL to obtain an aqueous CS solution. Finally, 2.00 mg of MoS₂/ZnO was dispersed in 4.00 mL of phosphate buffer (pH=7.6), 0.10/0.40/1.00 mL of CS solution was added dropwise under stirring (500 r·min⁻¹), and stirring was continued after the completion of dropwise addition for 1 h. Finally, the precipitate was collected by centrifugation (5000 × g, 20 min) and further cleaned by centrifugation with ultrapure water (5000 × g, 20 min) three times. The obtained MoS₂/ZnO@CS was preserved in the form of an aqueous dispersion, which was abbreviated as MZC-0.1, MZC-0.4, or MZC-1, according to the mass percentage of CS.

Similarly, ZnO@CS was prepared with a ZnO : CS mass ratio of 10 : 4.

2.4 Characterization of MoS₂/ZnO and MoS₂/ZnO@CS

SEM (GeminiSEM 500, Zeiss) was used for imaging at a high voltage of 200 kV for morphology analysis. The ratio of Mo to Zn in the samples was determined via ICP-AES (ICAP 7400, Thermo Fisher Scientific). To detect the presence of CS, an FT-IR spectrometer (Nicolet 6700, Thermo Fisher Scientific) was used. The zeta potential of the nanoparticles was determined via a highly sensitive zeta potential and particle size analyzer (NanoBrook 90Plus PALS, Brookhaven). A fluorescence spectrophotometer (F-4600, Hitachi) was used to obtain photoluminescence spectra, with excitation light of 200 nm or 300 nm and slit widths of 5 nm for both excitation and emission light. A UV-Vis spectrophotometer (UV-3600 Plus, Shimadzu) with a slit width of 5 nm was used to calculate the band gap energy. To determine the presence of MoS₂ and ZnO crystals, lattice streak analysis was performed via high-resolution TEM (JEM-2100 Plus, JEOL) under high voltage at 200 kV. XRD data of MoS₂/ZnO were obtained via an X-ray diffractometer (SmartLab SE, Rigaku).

2.5 Acid titration experiment with ZnO and CS aqueous dispersions

Aqueous dispersions of 50.00 mL of ZnO (0.01 M) and CS (0.02 M amino groups) were prepared. A 0.10 M HCl solution was subsequently added gradually, and the pH change was recorded. As a control, a 0.10 M HCl solution was added gradually to 50.00 mL of ultrapure water, and the change in pH was recorded. Notably, when the CS aqueous dispersion

was prepared, the CS was first dissolved with an appropriate amount of HCl solution, and then the NaOH solution was slowly added until a flocculent precipitate formed. Finally, the solution was diluted to the desired concentration with water.

2.6 Dissolution experiment

A solution with a pH range of 1.0–13.0 was prepared using HCl and NaOH. This solution was then used to disperse MoS₂/ZnO, MZC-0.1, MZC-0.4, and MZC-1. The final concentration of the aqueous dispersions was 32 µg·mL⁻¹ (in MoS₂/ZnO dose). Then, these aqueous dispersions were placed in centrifuge tubes and shaken at 200 r·min⁻¹ for 1 h. After centrifugation (10000 × g, 20 min), the undissolved precipitate was removed, and the concentrations of Mo and Zn in the supernatant were determined via ICP–AES.

The method was repeated using aqueous solutions of varying pH to disperse ZnO or ZnO@CS. The final concentrations of the ZnO or ZnO@CS aqueous dispersions were 500 µg·mL⁻¹ and 1 µg·mL⁻¹ (in ZnO dose).

2.7 ROS assay

To investigate whether MoS₂/ZnO@CS can produce ROS in the presence of light, we used DCFH as a fluorescent probe, which emits fluorescence when oxidized to DCF by ROS^[32]. First, 0.40 mL of NaOH aqueous solution (0.01 M) was added to 0.10 mL of DCFH-DA solution (1.00 mM in DMSO). The resulting mixture was allowed to stand in a dark environment for 30 min. Next, 2.00 mL of an aqueous sodium dihydrogen phosphate solution (25.00 mM) was added to obtain a 0.04 mM DCFH solution. The MoS₂/ZnO@CS, ZnO@CS, and DCFH solutions were sequentially added to PBS buffer. The final concentration of MoS₂/ZnO@CS (in MoS₂/ZnO dose) and ZnO@CS (in ZnO dose) was 32 µg·mL⁻¹, and the concentration of DCFH was 0.02 mM. The total volume was 0.20 mL. The aqueous dispersions were placed under simulated solar irradiation for 10 min, followed by centrifugation at 10000 × g for 10 min to remove the precipitate. Fluorescence emission spectra were obtained via a fluorescence spectrophotometer (F-4600, Hitachi, λ_{ex}/λ_{em} = 485 nm/500–700 nm, with a slit width of 5 nm for both excitation and emission wavelengths). The control group did not have any nanoparticles added, and the DCFH concentration was 0.02 mM.

In addition, we used ascorbic acid (AA) as another ROS probe, which has UV absorption at 266 nm, but dehydroascorbic acid, a product oxidized by ROS, has no UV absorption^[33,34]. Aqueous dispersions of MoS₂/ZnO@CS or ZnO@CS and an ascorbic acid solution were added to PBS buffer. The final concentrations of MoS₂/ZnO@CS (in MoS₂/ZnO dose) and ZnO@CS (in ZnO dose) were 32 µg·mL⁻¹, and the concentration of AA was 0.06 mM, with a total volume of 0.20 mL. The resulting aqueous dispersions were left under simulated solar irradiation for 10 min, followed by centrifugation at 10000 × g for 10 min to remove the precipitate, and the UV–Vis spectra of the supernatants were obtained via a UV–Vis spectrophotometer (UV-3600 Plus, Shimadzu). The control group did not have any nanoparticles added and had an AA concentration of 0.06 mM.

2.8 Bacterial plate-killing assay

The antibacterial efficiency of MoS₂/ZnO@CS was evaluated via a plate-killing assay. Four laboratory model strains were selected: *Escherichia coli* (ATCC 25922, gram-negative), *Staphylococcus aureus* (ATCC 25923, gram-positive), *Bacillus subtilis* (ATCC 6051, gram-positive), and *Pseudomonas aeruginosa* (ATCC 2785, gram-negative). Notably, *E. coli*, *S. aureus* and *P. aeruginosa* are typical bacteria in water mentioned in the “Guidelines for Drinking Water Quality: Fourth Edition Incorporating the First and Second Addendum” published by the World Health Organization^[35]. Moreover, *E. coli* and *P. aeruginosa* are gram-negative bacteria, whereas *S. aureus* is a gram-positive bacterium. To provide a more comprehensive understanding of the broad-spectrum antibacterial efficacy of MoS₂/ZnO@CS, an additional gram-positive bacterium, *B. subtilis*, was included in this study.

The cryopreserved bacterial suspension was thawed and inoculated onto TSB agar plates via the streak plate method, after which the agar plates were placed in a bacterial incubator (37 °C, 200 r·min⁻¹) until individual colonies appeared. The agar plates were stored in a 4 °C refrigerator for backup. At the start of the experiment, we conducted a primary inoculation by taking 3–5 individual colonies from the agar plates. We used an inoculation loop to transfer the colonies into 2.50 mL of fresh sterile tryptone soy broth (TSB) medium. The medium was then placed in a bacterial incubator (37 °C, 200 r·min⁻¹) and cultured for 16–18 h until bacterial growth reached the stationary phase. Next, a secondary inoculation was performed by adding 0.04 mL of the bacterial suspension from the primary inoculation medium to 4.00 mL of fresh sterile TSB medium. The mixture was then incubated in a bacterial incubator (37 °C, 200 r·min⁻¹) until the logarithmic phase was reached (OD₆₀₀ = 0.5–0.7), which took approximately 1–2 h. The bacteria were subsequently harvested by centrifugation (10000 × g, 5 min, 4 °C) and washed twice by centrifugation (10000 × g, 5 min, 4 °C) with sterile PBS, and the bacterial concentration was diluted to approximately 1.5 × 10⁶ CFU·mL⁻¹.

To study the disinfection efficiency of sunlight, 0.05 mL of diluted bacterial suspension and 0.10 mL of sterile water were added to 96-well plates, which were subsequently placed under simulated sunlight (0.1 W·cm⁻²) for 0 min, 2 min, 4 min, 8 min, 12 min, or 20 min, after which they were placed in a dark environment for 2 h. Next, serial 10-fold dilutions were subsequently made with sterile PBS buffer, followed by plating the dilutions (0.02 mL) onto TSB agar plates overnight incubation at 37 °C to form visible colonies. The colonies were counted, and the bacterial survival ratio was calculated by comparison with that of the group that did not receive irradiation.

The same methodology was used to study the antibacterial efficiency of antibacterial nanoparticles under sunlight for 10 min. A 0.05 mL diluted bacterial suspension and 0.10 mL aqueous dispersion of prediluted nanoparticles were added to a 96-well plate so that the final concentration of the nanoparticles was 32 µg·mL⁻¹ (in MoS₂/ZnO, ZnO or MoS₂ dose).

2.9 Live/dead staining of *E. coli*

E. coli (ATCC 25922, gram-negative) was stained with a bacterial live/dead viability kit. SYTO-9 and propidium iodide (PI) are two nucleic acid stains with different spectral characteristics and bacterial membrane penetration abilities. SYTO-9 can penetrate the cell membrane, is used to label RNA and DNA in live and dead cells, and is suitable for the staining of various bacteria. However, it can penetrate the cell membrane of damaged or dead cells, making their nuclei appear red^[36,37]. *E. coli* in the logarithmic growth phase were obtained according to the “Bacterial plate-killing assay”, and the bacterial concentration was diluted to approximately 1.5×10^8 CFU/mL. A 0.05 mL diluted bacterial suspension and 0.10 mL aqueous dispersion of prediluted nanoparticles were added to a 96-well plate so that the final concentration of the nanoparticles was $32 \mu\text{g} \cdot \text{mL}^{-1}$ (in MoS₂/ZnO, ZnO or MoS₂ dose). After being placed under simulated sunlight ($0.1 \text{ W} \cdot \text{cm}^{-2}$) for 10 min, 5 μL of SYTO-9 (0.19 mM) and 5 μL of PI (0.25 mM) were added to 0.10 mL of the treated bacterial suspension, which was then placed in the dark for 15 min for staining. Free SYTO-9 and PI were then removed via centrifugation ($10000 \times g$, 5 min), and the mixture was washed twice with PBS. A 0.01 mL bacterial suspension was dropped onto a glass slide and placed under a laser scanning confocal microscope (LSM880, Zeiss) for imaging.

2.10 SEM imaging of *E. coli*

The “Bacterial plate-killing assay” was used to obtain *E. coli* at the logarithmic phase, and the concentration of bacteria suspension was diluted to approximately 1.5×10^8 CFU/mL. Then, 0.05 mL of the diluted bacterial suspension and 0.10 mL of the aqueous dispersion of prediluted MoS₂/ZnO@CS were added to a 96-well plate such that the final concentration of the nanoparticles was $32 \mu\text{g} \cdot \text{mL}^{-1}$ (in MoS₂/ZnO dose). After being placed under simulated sunlight ($0.1 \text{ W} \cdot \text{cm}^{-2}$) for 10 min, the bacterial suspension was fixed with 4% paraformaldehyde at 4 °C for 1 h, dehydrated with a series of graded ethanol solutions (25%, 50%, 75%, 90%, and 100%) via centrifugation ($10000 \times g$, 5 min), and dispersed in 0.10 mL of 100% ethanol. Approximately 0.01 mL of the resulting dispersion was dropped onto a copper tape and left to dry. The dried sample was then sputtered with gold and imaged via SEM (GeminiSEM 500, Zeiss).

3 Results and discussion

3.1 Preparation and characterization of MoS₂/ZnO@CS

The application of pure ZnO as a photocatalyst is limited because of the high rate of recombination of photogenerated electron-hole pairs. Several strategies have been investigated to increase electron-hole separation, such as combining photocatalysts with other semiconductors, carbon materials, or noble metals^[15]. MoS₂ is a two-dimensional layered material with a direct band gap energy of a single layer of approximately 1.8–1.9 eV^[38]. It can be combined with large bandgap energy semiconductors such as ZnO to form heterojunctions in the interfacial region, which enhances the spatial separation of electron-hole pairs, thereby improving the photocatalytic efficiency of ZnO^[39].

MoS₂ nanoparticles with a flower-like morphology were synthesized via a hydrothermal method, resulting in a high surface area suitable for ZnO growth (Fig. 1a). Subsequently, ZnO was grown in situ on the MoS₂ nanoparticles using zinc acetate as the zinc source, resulting in the formation of MoS₂/ZnO composites (Fig. 1b). High-resolution transmission electron microscopy (HRTEM) and X-ray diffraction (XRD) were used to determine the presence of MoS₂ and ZnO in the composites (Figs. S1 and S2 in Supporting information). The mass ratio of MoS₂ to ZnO was found to be approximately 100 : 24 via ICP-AES. As a photocatalyst, light absorption is necessary for the production of charge carriers. Therefore, we characterized the optical properties of MoS₂/ZnO. The photoluminescence (PL) spectra of MoS₂, ZnO, and MoS₂/ZnO were investigated since the recombination of photogenerated carriers produces spectral radiation that causes PL in the materials. Under optical excitation at a wavelength of 200 nm, the PL spectrum of ZnO peaks at 405 nm (Fig. S3a). Similarly, MoS₂ exhibits a peak in its PL spectrum at 610 nm under optical excitation (Fig. S3b). However, in the case of MoS₂/ZnO, the peak is significantly weakened, indicating weakened photogenerated charge carrier recombination compared with that of pure MoS₂ or ZnO. This indicates that an increasing number of carriers will be engaged in the photocatalytic reaction. UV-Vis spectroscopy can be used to calculate the bandgap energy of a semiconductor by plotting $(\alpha h\nu)^2$ versus $h\nu$ ^[40]. In this study, the band gap energy of MoS₂/ZnO was found to be 2.72 eV (Fig. S4), which is lower than that of ZnO and is consistent with previous literature^[39]. This allows for a wider range of the solar spectrum to be utilized.

To address the question of how to combine CS with MoS₂/ZnO, we were inspired by the nanoprecipitation method of PLGA nanoparticle preparation^[41,42]. We determined that CS is soluble in acidic solutions and insoluble in alkaline solutions. First, we dissolved CS in dilute HCl and adjusted the pH to approximately 5.0. Then, accompanied by rapid stirring, the solution was slowly dripped into the MoS₂/ZnO dispersed phosphate buffer (pH = 7.6). In this process, the solution containing CS was transferred from a good solvent to a bad solvent, resulting in rapid precipitation, with the dispersed phase MoS₂/ZnO acting as the nucleation site, which was termed MoS₂/ZnO@CS (Fig. 1g). Scanning electron microscopy (SEM) revealed that the MoS₂/ZnO nanoparticles were encapsulated by a new layer of material (Fig. 1c). Additionally, energy-dispersive X-ray spectroscopy (EDS) mapping revealed the presence of Mo, S, Zn, O, and C (Fig. 1e). Notably, C is a characteristic element of chitosan. Moreover, Fourier transform infrared spectroscopy (FT-IR) of MoS₂/ZnO@CS revealed the characteristic peaks of CS (Fig. 1e). Since chitosan has a positively charged amine, the zeta potential shifts from -24.23 mV to 48.95 mV (Fig. 1f). These results suggest that the preparation of MoS₂/ZnO@CS was successful. Furthermore, to determine the stability of MoS₂/ZnO@CS, we tested the release of metal ions when the material was kept in an aqueous dispersion for a long period of time. The results showed that MoS₂/ZnO@CS was well stabilized and did not release large amounts of Mo and Zn ions in water due to hydrolysis within 10 d (Fig. S5).

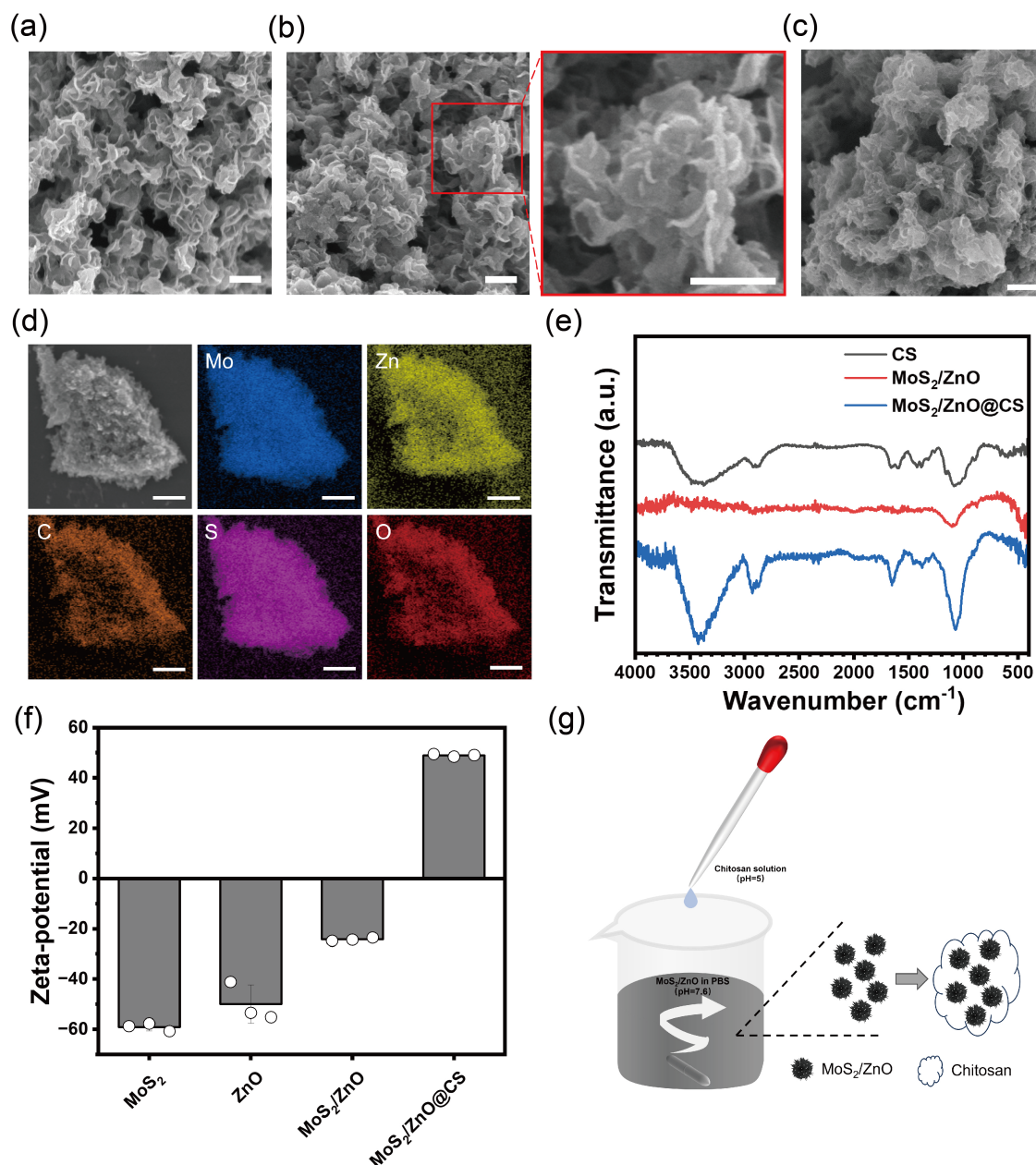


Fig. 1. Preparation and characterization of MoS₂/ZnO@CS. (a) SEM images of MoS₂, (b) MoS₂/ZnO and (c) MoS₂/ZnO@CS; scale bar = 200 nm. (d) EDS mapping of MoS₂/ZnO@CS; scale bar = 5 μ m. (e) FT-IR spectra. (f) Zeta potential. (g) Scheme of the precipitation method used to encapsulate the chitosan in MoS₂/ZnO.

3.2 Dissolution of MoS₂/ZnO@CS in water

Three types of MoS₂/ZnO@CS with MoS₂/ZnO and CS mass ratios of 10 : 1, 10 : 4, and 10 : 10 were prepared by varying the amount of CS, abbreviated as MZC-0.1, MZC-0.4, and MZC-1, respectively. We investigated the ability of CS encapsulation to inhibit the dissolution of photocatalysts by dispersing MoS₂/ZnO@CS in HCl or NaOH solutions with different initial pH values at a concentration of 32 μ g·mL⁻¹ (in MoS₂/ZnO dose). After shaking for 1 h, the mixture was centrifuged to remove the precipitate. The concentrations of Zn and Mo in the supernatant were detected via ICP-AES. More than 50% of the mass of ZnO was soluble at pH $\leq$ 3.0 or pH $\geq$ 13.0, regardless of its encapsulation with or without CS,

whereas the dissolution of ZnO in MZC-0.4 and MZC-1 decreased in the range of pH = 5.0–11.0, suggesting that the presence of CS can inhibit the dissolution of ZnO (Fig. 2a). In the case of MoS₂, dissolution is minimal and only occurs at pH=13.0. The presence of CS also inhibits its dissolution (Fig. 2b).

The above results indicate that the stability of ZnO in water is much lower than that of MoS₂. Therefore, we aimed to study the protective properties of chitosan against ZnO nanoparticles separately to verify the generalizability of the results. Nano-ZnO was encapsulated by CS at a 10 : 4 mass ratio to prepare ZnO@CS. SEM and EDS mapping images revealed that the CS successfully encapsulated the nano-ZnO

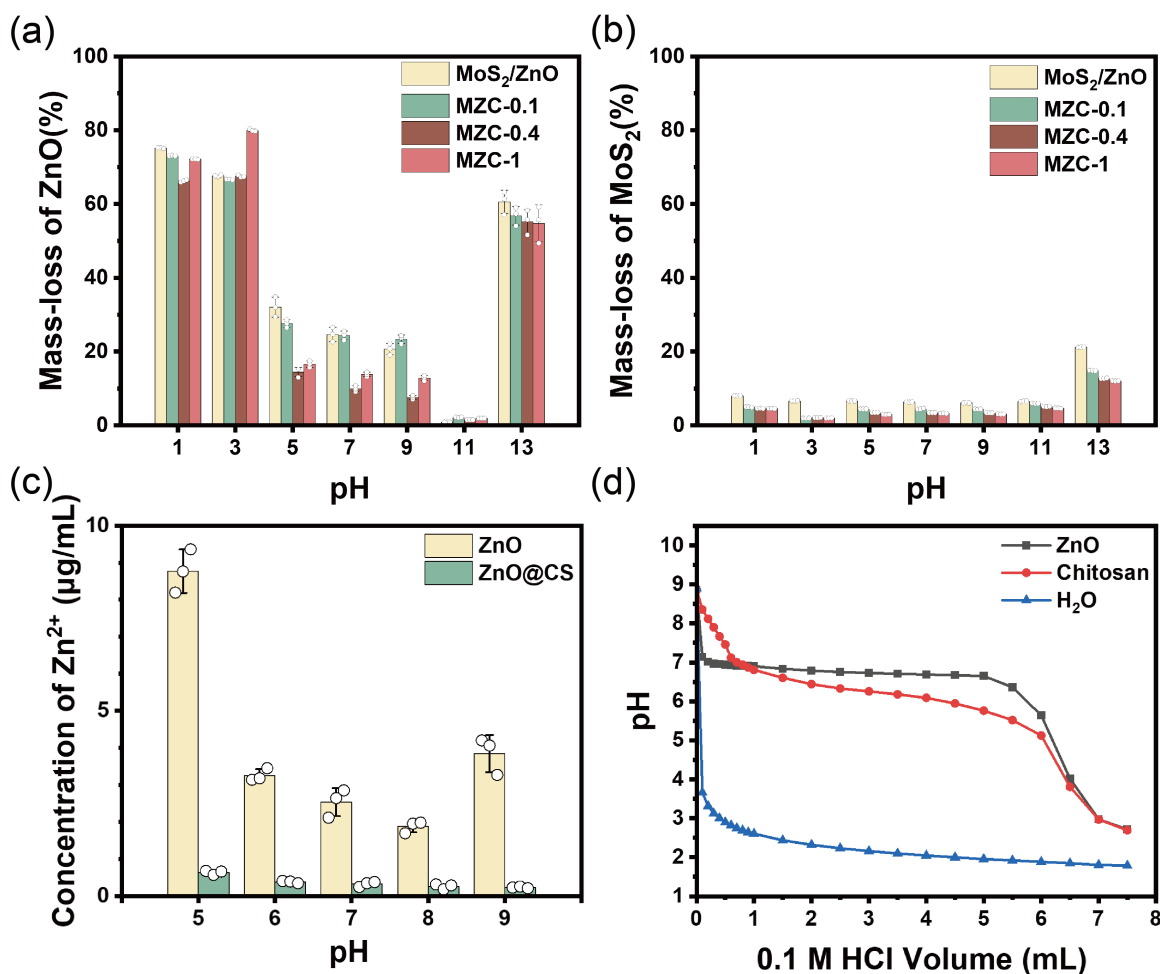


Fig. 2. Dissolution of MoS₂/ZnO@CS in water. (a) Mass loss of ZnO and (b) MoS₂ by dispersing MoS₂/ZnO and different amounts of CS from MoS₂/ZnO@CS in aqueous solutions at different pH values for 1 h at a concentration of 32 μg·mL⁻¹ (in MoS₂/ZnO dose). (c) Zn²⁺ concentration in the supernatant when ZnO and ZnO@CS were dispersed in aqueous solutions of different pH values for 1 h at a concentration of 500 μg·mL⁻¹ (in ZnO dose). (d) Changes in the pH of an aqueous dispersion of ZnO (0.01 M, 50.00 mL), an aqueous dispersion of CS (0.02 M of amino groups, 50.00 mL), and ultrapure water (50.00 mL) upon slow addition of HCl (0.10 M).

particles (Fig. S6). The dissolution of ZnO and ZnO@CS was investigated in different pH environments. At concentrations as low as 1 μg·mL⁻¹, the dissolution of ZnO was significantly affected by pH (Fig. S7a). Specifically, more than 50% of the ZnO dissolved at pH ≤ 4.0. At the same pH, ZnO@CS dissolved less than ZnO did, but the difference was not significant. However, when the concentration of ZnO was as high as 500 μg·mL⁻¹, the dissolution effect of CS was more effective (Fig. S7b). Additionally, the Zn²⁺ concentration in the supernatant of the ZnO@CS group was much lower than that of the ZnO group, measuring less than 1 μg·mL⁻¹ at pH = 5.0–9.0 (Fig. 2c).

To determine why chitosan inhibits the dissolution of ZnO, an acid titration experiment was performed (Fig. 2d). A nano-ZnO aqueous dispersion (0.01 M, 50.00 mL) with an initial pH of approximately 8.9 was used. Upon the addition of 0.10 mL of HCl (0.10 M), the pH rapidly decreased to 7.1 and continued to decrease slowly with the addition of HCl (0.10 M). The section of the curve with a lower slope indicates the pH at which the reaction between ZnO and H⁺ reaches equilibrium, which is between approximately 6.7 and 7.1. We

subsequently investigated the pH change in the aqueous dispersion of CS (0.02 M amino groups, 50.00 mL) and ultrapure water (50.00 mL) upon the addition of HCl (0.10 M). The pH of the CS solution changed at a slow rate, even after the addition of 0.60 mL of HCl (0.10 M), resulting in a pH value of approximately 7.5, which remained higher than the pH at which the reaction between ZnO and H⁺ reached equilibrium. This result indicated that CS has potential as a protective agent to inhibit ZnO dissolution.

3.3 ROS generation through MoS₂/ZnO@CS photocatalysis

As a key antibacterial mechanism, metal oxide nanoparticles utilize the oxidative stress induced by reactive oxygen species (ROS)^[43,44]. In this study, we investigated the ROS-generating ability of MoS₂/ZnO using 2',7'-dichlorodihydrofluorescein (DCFH) and ascorbic acid (AA) as ROS probes. DCFH is a fluorescent probe that measures ROS levels and fluoresces strongly when oxidized by ROS to 2',7'-dichlorofluorescein (DCF)^[32]. Ascorbic acid (AA) exhibits UV absorption at 266 nm but not after oxidation to dehydroascorbic acid

by ROS^[33,34]. The results of these two methods revealed that both MoS₂/ZnO@CS and ZnO@CS produced ROS. Owing to the distinctive optical properties of MoS₂/ZnO, MoS₂/ZnO@CS generated more ROS than did ZnO@CS at the same concentration (Fig. 3a, b).

Considering that CS can inhibit the dissolution of MoS₂/ZnO, we investigated the production of ROS at different pH values. The DCFH fluorescent probe did not produce fluorescence emission peaks at pH ≤ 2.0. However, the peak intensity at 525 nm gradually increased with increasing pH. Notably, the peak of MoS₂/ZnO@CS was higher than that of MoS₂/ZnO (Fig. 3c). This is because, even with the same amount of MoS₂/ZnO feeding, the loss of MoS₂/ZnO in MoS₂/ZnO@CS is less due to dissolution.

One concern is whether ROS lead to CS decomposition and

thus the release of metal ions. The review article by Cao et al.^[45] summarized the representative ROS-responsive moieties of polymers. On the basis of these findings, chitosan is a polysaccharide that does not have ROS-sensitive chemical bonds in its molecular structure. In addition, we investigated the release of Mo⁴⁺ and Zn²⁺ from MoS₂/ZnO@CS after long-term light exposure (Fig. S8). The experimental results revealed that there was no substantial increase in the release of Mo⁴⁺ and Zn²⁺ under 2 h of continuous light. Therefore, it can be reasonably concluded that ROS do not result in the decomposition of CS.

3.4 Photocatalytic antibacterial action of MoS₂/ZnO@CS under simulated solar irradiation

The experimental results above demonstrated that

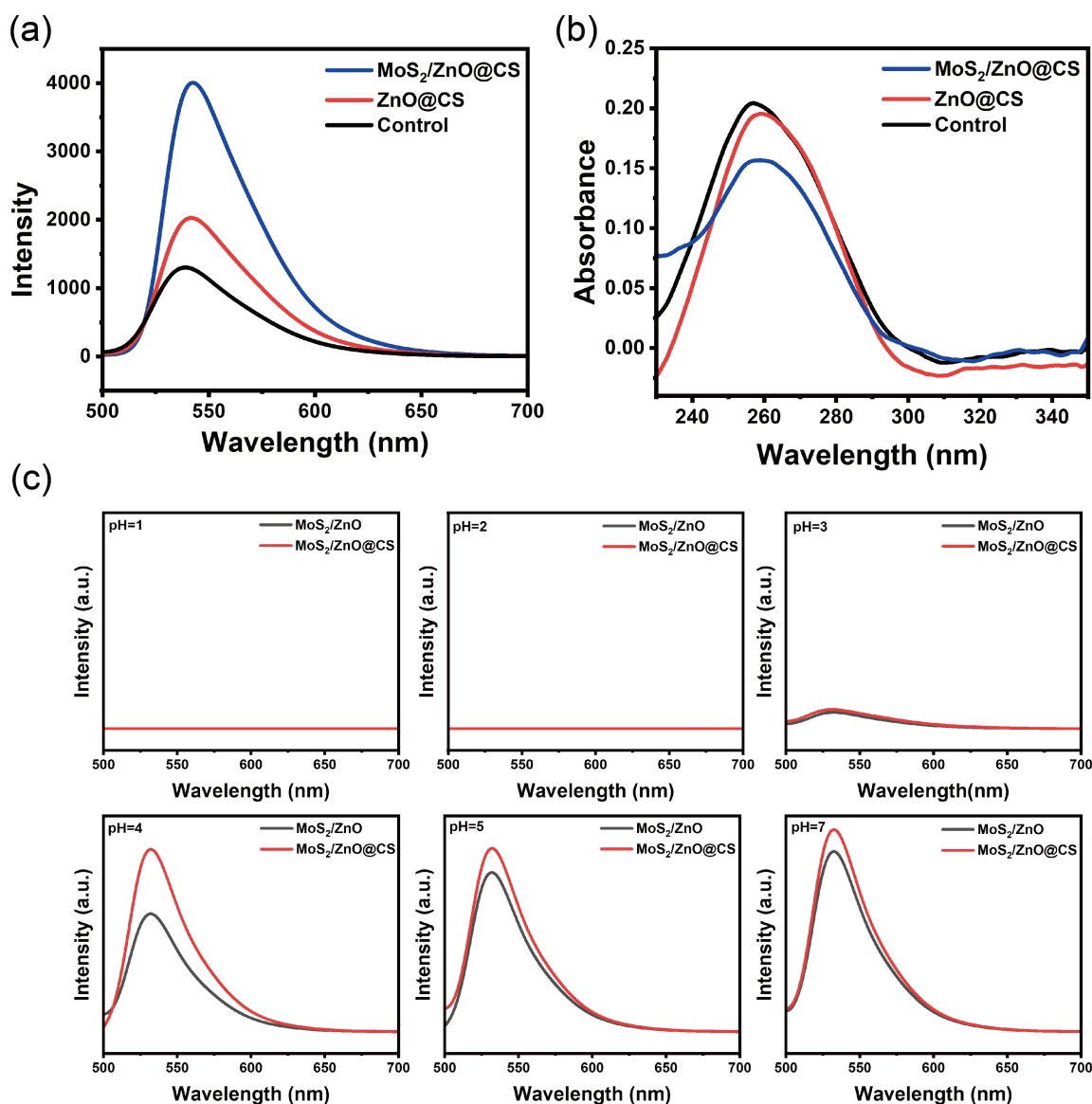


Fig. 3. ROS generation through MoS₂/ZnO@CS photocatalysis. (a) Fluorescence spectra of DCFH and (b) UV-Vis absorption spectra of AA after the addition of MoS₂/ZnO@CS or ZnO@CS at a concentration of 32 μg·mL⁻¹ (in MoS₂/ZnO or ZnO dose) and irradiation (0.1 W·cm⁻²) for 10 min. (c) Fluorescence spectra of DCFH after the addition of MoS₂/ZnO@CS or MoS₂/ZnO at a concentration of 32 μg·mL⁻¹ (in MoS₂/ZnO dose) and irradiation (0.1 W·cm⁻²) for 10 min in solutions of different pH values.

MoS₂/ZnO@CS has low dissolution and high ROS production under light, which is expected to be a water disinfectant in solar disinfection (SODIS). Ultraviolet (UV) light has been used to inactivate a wide range of infectious organisms, and advances made in recent decades have shown that SODIS is an effective method of providing water that can significantly reduce morbidity and mortality from waterborne diseases^[46]. To verify the bactericidal effect of solar irradiation, we investigated the survival ratios of *E. coli* (gram-negative), *S. aureus* (gram-positive), *B. subtilis* (gram-positive), and *P. aeruginosa* (gram-negative) under simulated solar irradiation (0.1 W·cm⁻²). Bacterial suspensions with a concentration of approximately 5×10⁵ CFU/mL were used to simulate water contaminated with bacteria^[47,48]. We placed 0.15 mL of bacterial suspension in a microtiter plate with a bottom area of 0.32 cm². The antibacterial efficiency against these four typical bacteria was investigated via the plate count method. The experimental results indicate that sunlight irradiation has an antibacterial effect to some extent and that different bacteria have varying degrees of sensitivity to it. After being exposed to light for 20 min, *S. aureus* only had a survival ratio of 26.08% (Fig. 4a, b).

To increase the effectiveness of sunlight for sterilization, MoS₂/ZnO@CS was added to the experimental conditions described above, and the light irradiation time was set to 10 min (Fig. 4c, d; Figs. S9–S12). According to the experimental results, the antibacterial efficacy of MoS₂ has been observed to be relatively low, even compared to that of light irradiation alone. MoS₂ absorbs UV light that would otherwise have antibacterial activity but radiates energy outward due to photo-generated electron-hole pair recombination instead of generating ROS. Owing to the unique optical properties of MoS₂/ZnO, more ROS can be generated, and its antibacterial efficacy is approximately 10 times greater than that of ZnO, resulting in 99.99% (*E. coli*), 99.98% (*S. aureus*), 99.92% (*B. subtilis*), and 99.95% (*P. aeruginosa*) sterilization ratios for the four types of bacteria. Notably, when MoS₂/ZnO was encapsulated with CS, there was a slight decrease in antibacterial efficiency with increasing mass percentage of CS, which was attributed to the fact that excessive CS inhibited the loss of MoS₂/ZnO due to dissolution but also affected the release of ROS. However, this inhibitory effect on ROS release does not seriously impede sterilization as long as the mass percentage of CS encapsulated is moderate. The use of cell membranes and biocompatible polymers to encapsulate photodynamic nanoparticles to increase their biocompatibility or targeting ability is a widely used approach in the field of nanomedicine^[49–52]. In addition, CS encapsulation transforms the zeta potential of MoS₂/ZnO to a positive value (Fig. 1f), whereas the majority of bacteria have negative zeta potentials^[53], which favor contact between particles and bacteria through electrostatic interactions. Bacteria are more accessible to the MoS₂/ZnO@CS surface and are attacked by freshly generated ROS, thus killing bacteria even in the absence of free diffusion of ROS. To verify this adsorption, cosedimentation experiments between particles and bacteria were conducted because electrostatic adsorption of particles and bacteria may lead to larger sizes that are more susceptible to settling. MoS₂/ZnO@CS was homogeneously mixed

with *E. coli* and allowed to stand. The supernatant was gently aspirated at intervals, and the OD₆₀₀ was measured, which represented the total concentration of particles and bacteria in the supernatant. The results showed that the OD₆₀₀ of the mixture of MoS₂/ZnO@CS and *E. coli* was already lower than that of the control group of *E. coli* after 30 min, and the OD₆₀₀ continued to decrease with time (Fig. S13a), which implies that cosedimentation of MoS₂/ZnO@CS and *E. coli* occurred due to adsorption. The precipitates were imaged via SEM (Fig. S13b). *E. coli* was attached to MoS₂/ZnO@CS, which validated the occurrence of electrostatic adsorption of MoS₂/ZnO@CS and *E. coli*. On the basis of the above results, controlling the mass ratio of MoS₂/ZnO to CS to approximately 10 : 4 can maintain high antibacterial activity while imparting low dissolution of MoS₂/ZnO@CS. In addition, this photocatalytic antimicrobial effect was concentration dependent. When the concentration was reduced from 32 µg·mL⁻¹ to 4 µg·mL⁻¹, the sterilization ratio of MZC-0.4 decreased from 99.98% (*E. coli*), 99.97% (*S. aureus*), 99.92% (*B. subtilis*), and 99.91% (*P. aeruginosa*) to 99.50% (*E. coli*), 99.81% (*S. aureus*), 99.43% (*B. subtilis*), and 99.50% (*P. aeruginosa*).

To demonstrate that the bactericidal effect is primarily due to the ROS produced in the light, we investigated the survival of bacteria in the dark (Fig. 4e, f; Figs. S9–S12). The results show that the sterilization ratio is reduced in the absence of light. The antibacterial efficiency of ZnO was slightly greater than that of MoS₂/ZnO at this time, and the encapsulation of CS attenuated the antibacterial efficiency. On the basis of these results, we propose that the antibacterial efficiency without light irradiation is due to the release of Zn²⁺ resulting from the partial dissolution of ZnO^[54]. Research has demonstrated that Zn²⁺ can be adsorbed onto bacterial surfaces, leading to interactions with bacterial cell membranes. This interaction can facilitate the entry of Zn²⁺ into bacteria and their interaction with functional groups such as sulfhydryl, amino, and hydroxyl groups on biologically active proteases. This can alter the structure and performance of proteases, ultimately causing bacterial death due to metabolic imbalance^[55].

To visualize the death of the bacteria, *E. coli* was stained with SYTO-9 and PI. SYTO-9 is a green fluorescent nucleic acid dye that can penetrate cell membranes and stain RNA and DNA in living and dead eukaryotic cells, as well as gram-positive and gram-negative bacteria. Another dye is PI, a nuclear stain that binds to DNA and fluoresces red. PI is unable to penetrate intact cell membranes but can penetrate broken cell membranes in late apoptotic and dead cells, resulting in red staining of the nucleus^[36,37]. In the control group without light, *E. coli* showed minimal death. After light treatment, partial bacterial death occurred, while a significant number of bacteria were killed after treatment with MoS₂/ZnO@CS under light (Fig. 4g). The attack of ROS leads to the loss of cell membrane potential, resulting in an increase in cell membrane permeability. This is followed by the leakage of cytoplasmic contents such as proteins and DNA, leading to rapid decay and ultimately cell death^[56]. This morphological change in *E. coli* was verified through SEM (Fig. 4h, i).

4 Conclusions

In conclusion, this work achieved the encapsulation of

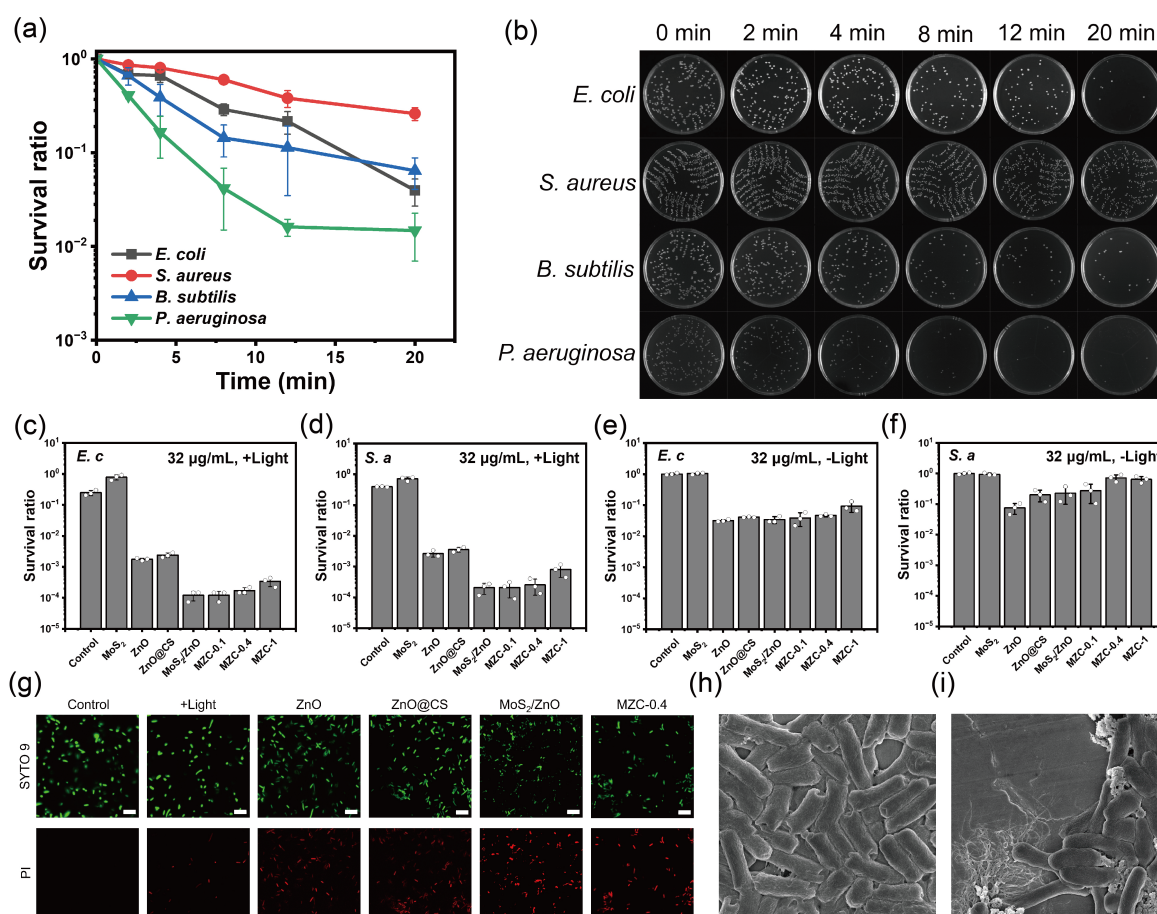


Fig. 4. Antibacterial action of MoS₂/ZnO@CS. (a) Survival ratios of *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa* under simulated solar illumination (0.1 W·cm⁻²) over time. (b) Corresponding agar plate images. (c–f) Survival ratios of *E. coli* and *S. aureus* with or without simulated solar illumination (0.1 W·cm⁻²) after the addition of different nanoparticles at a concentration of 32 µg·mL⁻¹ (in MoS₂/ZnO, ZnO or MoS₂ dose). (g) Images of live/dead staining of *E. coli* with nanoparticle concentrations of 32 µg·mL⁻¹ (in MoS₂/ZnO, ZnO or MoS₂ dose); scale bar = 10 µm. (h) SEM image of *E. coli* without any treatment; scale bar = 1 µm. (i) SEM image of *E. coli* after treatment with 32 µg·mL⁻¹ (MoS₂/ZnO, ZnO or MoS₂) of MZC-0.4 under simulated solar irradiation (0.1 W·cm⁻²) for 10 min; scale bar = 1 µm.

MoS₂/ZnO, an efficient photocatalyst, by chitosan via a precipitation method. Chitosan encapsulation inhibited the dissolution of ZnO in water, making the photocatalyst more suitable for water disinfection. Additionally, sterilization experiments revealed a greater than 99.9% sterilization ratio against four typical bacteria. It is recommended that MoS₂/ZnO@CS-treated water be used as domestic water (e.g., washing) rather than drinking water for people living in the backward areas of developing countries, except in emergencies. However, as a proof of concept, research has been conducted in the laboratory to study the technical performance of materials, using defined water types and targeted organisms for experiments, which may not reflect the complexity of the real world.

Supporting information

The supporting information for this article can be found online at <https://doi.org/10.52396/JUSTC-2024-0050>. The supporting information includes thirteen figures and three additional methods.

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Conflict of interest

The authors declare that they have no conflict of interest.

Biography

Yutao Xie is currently pursuing a master's degree under the supervision of Prof. Lihua Yang at the University of Science and Technology of China. His research mainly focuses on the applications of antibacterial materials in water treatment.

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