Visible-Light-Driven Catalytic Disinfection of *Staphylococcus aureus* Using Sandwich Structure g-C₃N₄/ZnO/Stellerite Hybrid Photocatalyst

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A novel g-C₃N₄/ZnO/stellerite (CNZOS) hybrid photocatalyst, which was synthesized by coupled hydro thermal-thermal polymerization processing, was applied as an efficient visible-light-driven photocatalyst against *Staphylococcus aureus*. The optimum synthesized hybrid photocatalyst showed a sandwich structure morphology with layered g-C₃N₄ (doping amount: 40 wt%) deposited onto micron-sized ZnO/stellerite particles (ZnO average diameter: ~18 nm). It had a narrowing band gap (2.48 eV) and enlarged specific surface area (23.05 m²/g). The semiconductor heterojunction effect from ZnO to g-C₃N₄ leads to intensive absorption of the visible region and rapid separation of the photogenerated electron-hole pairs. In this study, CNZOS showed better photocatalytic disinfection efficiency than g-C₃N₄/ZnO powders. The disinfection mechanism was systematically investigated by scavenger-quenching methods, indicating the important role of H₂O₂ in both systems. Furthermore, h⁺ was demonstrated as another important radical in oxidative inactivation of the CNZOS system. In respect of the great disinfection efficiency and practicability, the CNZOS heterojunction photocatalyst may offer many disinfection applications.

Keywords: Water disinfection, *S. aureus*, g-C₃N₄/ZnO/stellerite, visible light irradiation, ROS

Introduction

Various semiconductor photocatalysts such as TiO₂ [1], MgO [2], and ZnO [3–6] were involved in disinfection strategies. Owing to their high stability, low cost, and superior oxidative capability for organisms under mild conditions, they have shown significant potential in disinfection techniques. However, owing to their wide band gaps, these semiconductor particles require the excitation of the UV light, which occupies only 4% of the incoming solar spectrum [7, 8]. Thus, the abundant visible irradiation in sunlight cannot be utilized to induce the disinfection effect. The development of new light-absorbing materials is required to improve the harvesting of sunlight as well as to enhance the disinfection efficiency after modification involving deposition of noble metals [9], and doping of cations [10] and anions [11] and graphene sheets [12].

Among these effective modifications, graphitic carbon nitrides (g-C₃N₄), a newly emerging carbon material that

has received sustained attention, efficiently harvests the visible light and can photodegrade organic pollutants. To date, studies on disinfection using g-C₃N₄ has been rare. It may be because the fast recombination of photogenerated electron-hole pairs of pure g-C₃N₄ limits its utility. The hybrid photocatalyst g-C₃N₄/ZnO (CNZO) with heterojunction structures has been found to lead to rapid photo-induced charge separation and a relatively slow charge recombination [13]. Furthermore, its relatively narrow band gap leads to efficient light harvesting within the visible-light region and electric conductivity [14]. Nevertheless, there is no previous study about CNZO composites used as a bactericidal agent. To improve the applicability of nanoparticles for water treatment and solve the problem of their agglomeration, natural zeolites were selected as promising hosts and stabilizers owing to their unique features, such as broad specific area, and high amount of hydrophobic and hydrophilic active sites [15, 16]. Owing to their high whiteness, perfect stability, and low cost, stellerites attract

considerable attention for their industrial applications. Nevertheless, no previous study about the synthesis of g-C₃N₄/ZnO/stellerite (CNZOS) composites using stellerites as supports was reported.

Staphylococcus aureus, a gram-positive bacterium, represents a process indicator in water to test photocatalytic inactivation performance. So far, the mechanisms behind the disinfection by nanoparticles were membrane disorganization and reactive oxygen species (ROS). In the process of photocatalytic disinfection, the cell membrane of bacteria is attacked and damaged by ROS as well as the photogenerated electrons and holes under UV/visible irradiation [17–19]. Furthermore, bacterial growth is inhibited after that process. However, the visible-light-driven photocatalytic inactivation mechanism by ROS in the CNZOS systems is uncertain. Herein, we demonstrate the simple, self-assembled synthesis of CNZOS composites using as-prepared g-C₃N₄ nanosheets and ZnO/stellerite. The photocatalysts were characterized by SEM, XRD, UV-Vis, and BET analyses. The photocatalytic activity was evaluated by photocatalytic disinfection of *S. aureus* under visible-light irradiation (>420 nm). The CNZOS composites showed a much higher disinfection efficiency than CNZO and ZnO/stellerite under visible-light illumination. To prove the disinfection mechanisms by ROS in both CNZOS and CNZO systems, scavenger-quenching experiments were conducted.

Materials and Methods

Preparation of g-C₃N₄ Nanosheets

Original g-C₃N₄ was prepared by direct polymerization of melamine at high temperature. In detail, melamine was placed in an alumina crucible with a cover and then heated at 550°C for 4 h with a ramp rate of about 2.3°C/min for both the heating and cooling processes [20, 21].

Preparation of ZnO/Stellerite Composite and Pure ZnO

The synthesis of the ZnO/stellerite composite by a facile hydrothermal-calcination process has been listed as follows [5]. First, 100 ml of aqueous solution with zinc acetate (1.82 g) and urea (0.5 M) were added into the beaker, and then 4 g of stellerite powder was added as well. Next, the mixture was stirred continuously at 95°C for 4 h. Second, the mixture was vacuum-filtered and distilled water was added to adjust the pH of the mixture at 7.0. Finally, the powders were heated in an oven at 300°C for 2 h in air atmosphere to obtain ZnO/stellerite. The pure ZnO powder without carriers, used as reference, was also prepared.

Preparation of g-C₃N₄/ZnO/Stellerite and g-C₃N₄/ZnO Nanohybrids

First, 100 mg of ZnO/stellerite was dispersed in water (2 ml) by mild sonication for 2 min. As-prepared g-C₃N₄ nanosheet solutions

of different concentrations (2 ml; 10, 20, 30, 40, and 50 mg/ml) were added to the above suspension, leading to different weight percentages of g-C₃N₄ in the ZnO/stellerite (10, 20, 30, 40, and 50 wt%). Then, the precursors were stirred in the dark for 1 h, and washed thoroughly with distilled water three times for purification. Finally, the mixture was dried in a vacuum oven at 80°C for 2 h for further use [22]. The CNZOS composites were marked as CNZOS_x, where *x* indicates the mass ratio in the preparation, which was 10, 20, 30, 40, and 50, respectively. For comparison, different g-C₃N₄/ZnO samples without stellerite were also prepared with the same method and conditions.

Characterization

The crystal phase composition and crystallinity characteristics of the samples were determined using an X-ray diffraction diffractometer (XRD, Bruker D8 Advance). The surface morphologies of the synthesized photocatalysts were observed using a scanning electron microscope (SEM; Quanta 200; FEI, USA). Nitrogen adsorption and desorption isotherms as well as pore diameter distribution were recorded using a nitrogen adsorption apparatus (BET, JW-BK). UV-Vis absorption spectra were recorded on a Shimadzu UV-3150 device.

Bacterial Culture and Antibacterial Experiments

Monocolonies of *S. aureus* (Microbiology Chinese Academy of Science) on solid Luria–Bertani (LB) agar plates were transferred to 100 ml of liquid LB culture medium and grown at 37 °C for 12 h under 170 rpm rotation. Then, the bacteria were diluted in sterilized saline (0.9% NaCl) solution to 10⁷ CFU/ml [23]. The CNZOS_x samples were diluted in culture medium at concentrations of 100 mg/l by diluting the stock dispersion. ZnO/stellerite and CNZO nanohybrids were used with same particle content as a control. The as-prepared bacteria solution (10 ml) was mixed with pristine CNZOS_x, CNZO, or ZnO/stellerite nanohybrids for 2 h under visible-light illumination. The reaction temperature was maintained at 37°C under 170 rpm rotation. Then, the solution was placed on a solid medium by the spread-plate method and cultured at 37°C for 24 h. Further tests of the viability were conducted by observing the number of colony forming units on LB agar plates. Control experiments were performed in parallel without any catalysts.

Photocatalytic Mechanism

Furthermore, to prove directly the disinfection mechanism by ROS in the CNZOS systems, and to prove the carrier effect of stellerite, five scavenger-quenching experiments were conducted to study the disinfection contribution of each ROS as well as of the electrons and holes. Scavengers Cr(VI) (0.05 mM, 99.5%; Sigma, USA), TEMPO (1 mM, 99%; Sigma, USA), Fe(II)-EDTA (10 mM), isopropanol (0.5 mM; Sigma, USA), and sodium oxalate (0.5 mM, 99.5%; Sigma, USA) were used to quench e⁻, •O₂⁻, H₂O₂, OH•, and h⁺, respectively. These scavengers have been widely used to study the photocatalytic mechanism in bacterial inactivation [24–28].

Fig. 1. Scanning electron micrographs of (a) pure ZnO, (b) g-C₃N₄, (c) CNZO, (d) and CNZOS; and (e) EDS spectrum and element mapping of C, N, Zn, O, Si, and Al for the CNZOS composite.

Results and Discussion

Synthesis and Physical Characterization of the Hybrid Photocatalysts

The g-C₃N₄ nanosheets were synthesized from melamine powder according to a previously reported method with some modification [21]. The CNZOS nanohybrids were synthesized via a conventional solution impregnation method [29]. The detailed morphology and microstructure of the hybrid photocatalysts were imaged using SEM. Serious agglomeration of the particles was found from the SEM images of pure ZnO (Fig. 1a) and g-C₃N₄ (Fig. 1b). As Fig. 1b shows, the surface morphology of g-C₃N₄ is smooth, consisting of sheet-like structures rather than particles. The pure ZnO particles are well dispersed on the surface of g-

C₃N₄ (Fig. 1c). As found from Fig. 1d, some micro-aggregate particles appeared on the surface of the composites owing to the introduction of the CNZO particles. Thus, the ZnO particles on CNZOS are nano-sized particles and there are obvious differences among the size of ZnO nanoparticles (~17.8 nm–20.4 nm) after incorporating g-C₃N₄ onto the same system (Table 1). It was possibly attributed to the ZnO/stellerite precursors dispersed throughout the solution and dissolved g-C₃N₄ decreased the amount of ZnO precursor per unit volume and consequently affected the size of ZnO [30, 31]. In this case, the large stellerite acts as an excellent support to stabilize the formation of ZnO nanoparticles and g-C₃N₄ nanosheets. The presence of C, N, Si, Zn, Al, and O, shown in Fig. 1e, was measured by EDX under surface-distribution scanning. It clearly indicates the

Table 1. Morphological properties of the CNZOS_x and CNZO samples.

Sample	S _{BET} (m ² /g)	Pore volume (cm ³ /g)	Average pore radius (nm)	Crystallite size of ZnO (nm)
CNZOS ₁	10.195	0.017	22.546	20.417
CNZOS ₂	18.342	0.052	7.592	17.849
CNZOS ₃	19.606	0.055	7.511	20.023
CNZOS ₄	23.046	0.046	14.747	18.623
CNZOS ₅	39.059	0.023	24.165	19.231
CNZO	23.955	0.156	26.409	21.232

Fig. 2. XRD patterns of the samples. $g\text{-C}_3\text{N}_4$ (a); pure ZnO (b) and $g\text{-C}_3\text{N}_4/\text{ZnO/stellerite}$ composite powers: the $g\text{-C}_3\text{N}_4$ doping amount for (c)–(g) are 10%, 20%, 30%, 40%, and 50%, respectively.

good distribution of CNZO nanoparticles on the stellerite surface, although several of them tended to agglomerate. This result suggests that the current immobilized catalyst preparation method allows homogenous and intimate loading of CNZO nanoparticles onto stellerite to form a stable hybrid photocatalyst.

The characteristic diffraction peaks of the XRD pattern (Fig. 2) for the hybrid photocatalyst were as follows: $2\theta = 31.8, 34.4, 36.3, 47.6, 56.6, 62.9, 66.3, 68.0$, and 69.1 are

attributed to (100), (002), (101), (102), (110), (103), (200), (112), and (201) planes of ZnO. These data confirm the wurtzite structure of ZnO (JCPDS No. 36-1451) [22, 32]. The peak of 27.6° can be indexed to the (002) diffraction plane of $g\text{-C}_3\text{N}_4$ (JCPDS No. 87-1526), corresponding to the interlayer-stacking [33]. This confirms that ZnO particles were successfully grown during the hydrothermal process, and $g\text{-C}_3\text{N}_4$ sheets were well attached to the surface of ZnO/stellerite. The sharp and expansive diffraction peak revealed the high crystallization $g\text{-C}_3\text{N}_4$ grown in ZnO/stellerite composites.

Absorption spectra of CNZOS were employed to compare the band structure of stellerite, pure ZnO, $g\text{-C}_3\text{N}_4$, CNZO, and CNZOS $_x$ (Fig. 3A). Except for stellerite, the spectra were dominated by the ZnO and the $g\text{-C}_3\text{N}_4$ (the majority phase) light absorption capability in the UV and visible regions [34–37]. Among all the samples, pure ZnO has the strongest UV absorption and $g\text{-C}_3\text{N}_4$ has the strongest visible absorption. The presence of CNZOS was detected in the visible-light region, with the presence of a shoulder at the end of the sigmoidal decreasing shape at $\sim 380\text{--}420\text{ nm}$, the characteristic of the carbon nitride band gap. It was found that the optical absorption of CNZO is stronger than that of CNZOS. This can be attributed to the quantum confinement effect of the ZnO and $g\text{-C}_3\text{N}_4$ nanoparticles. The optical band gap of the as-prepared ZnO/stellerite, CNZO, and CNZOS nanohybrids were also investigated (Fig. 3B). CNZOS showed an absorption peak around 400 nm , which can be attributed to the absorption of $g\text{-C}_3\text{N}_4$ [26]. We summarized the band gap of the CNZO and CNZOS composite samples, presenting an average value of 2.92 eV and 3.18 eV , respectively. The difference of band

Fig. 3. (A) UV-Vis absorption spectra of stellerite, pure ZnO, CNZO, CN, and CNZOS $_x$ ($x = 1, 2, 3, 4, 5$); and (B) Tauc's plot of ZnO/stellerite, CNZO, and CNZOS $_4$.

gap between CNZO and CNZOS may be ascribed to the immobilization of stellerite. However, the band gap energy of 3.22 eV for ZnO/stellerite is similar to that of TiO₂ (3.2 eV) [1]. Both the CNZO and CNZOS composite samples show an intrinsic semiconductor-like absorption in the blue region of the visible spectrum. Apart from that, the value among CNZOS_x photocatalysts is essentially constant. All the facts indicate that g-C₃N₄ has a large influence in the optical properties of the major ZnO phase [38].

Table 1 summarizes the primary particle size of the ZnO component as well as the BET, pore size, and volume through the series. A large difference was seen from both CNZO and CNZOS samples with the ZnO size from ~17.8–21.2 nm. At the same time, the composite samples display, as expected, a nearly constant surface area (10.2–39.0 m²/g) and, in general, morphological properties (Fig. 1). They are thus dominated by the main ZnO component.

Photocatalytic Inactivation of *S. aureus*

To examine the antibacterial activity of the CNZOS nanohybrids, the G⁺ bacterium *S. aureus* was chosen as the model to evaluate the bactericidal performance and mechanism of disinfection. The bactericidal rates of the composites were evaluated in a sterile saline medium, and the results were determined by viable count measurements. The agents (CNZOS nanohybrids) at 100 mg/l with different g-C₃N₄ doping amounts were added to sterile saline medium and incubated for 2 h under visible-light irradiation.

The effect of the g-C₃N₄ doping amount in the CNZOS_x nanohybrids on the antibacterial activity was studied and the corresponding activities are summarized in Fig. 5A. The results demonstrated that after 2 h, for *S. aureus*, the CNZOS₄ nanohybrids exhibited strongest antibacterial activity. For the CNZOS₄ nanohybrids, the inactivation efficiency could reach nearly 90% and the disinfection rate

Fig. 4. Nitrogen adsorption-desorption isotherms of stellerite (A), ZnO/stellerite (B), CNZO (C), and CNZOS₄ (D), with corresponding pore-size distribution plot in the inset.

Fig. 5. (A) Inactivation efficiency of CNZOS_x ($x = 1, 2, 3, 4, 5$) under visible-light illumination using 100 mg/l of catalyst samples; and (B) disinfection-performance comparison of CNZOS₄ with CNZO, showing the high disinfection by CNZOS₄ after immobilization of the stellerite.

reached 0.98 h^{-1} ($R^2 = 0.99$), when the concentration was 100 mg/l under visible-light irradiation. Moreover, CNZOS_x effectively inhibited the growth of *S. aureus*, and the antibacterial activity was enhanced with increasing visible-light irradiation time. The photocatalytic disinfection rate of CNZOS_x increased with g-C₃N₄ doping from 10% to 40%, and with further increasing g-C₃N₄ doping to 50%, the disinfection rate decreased. According to Fig. 5A, *S. aureus* was almost completely killed within 2 h at a concentration of 100 mg/l. These results indicate that the CNZOS_x nanohybrids are an effective and rapid bactericidal agent even at low concentrations and with short illumination

times.

We also compared the bactericidal efficiency of the CNZOS nanohybrids and CNZO. The catalyst amount of the CNZO sample was 25 mg/l, which is equal to the CNZO content of 150 mg/l of CNZOS₄. Therefore, to understand the effect of catalyst composition on the antibacterial efficiency, CNZOS₄ varied from 50 to 100 mg/l (<150 mg/l) was compared with CNZO (25 mg/l) under visible illumination. The results are shown in Fig. 5B. The CNZOS₄ (100 mg/l) composite showed the highest disinfection activity than the other samples (50 and 75 mg/l) under visible light. About 75.53%, 89.73%, and 97.75% of

Fig. 6. Bacterial colonies of recultured *S. aureus* after treatment with CNZOS₄ (A) and CNZO (B) at different times.

S. aureus were inactivated by CNZOS₄ at 50, 75, and 100 mg/l, respectively, for 120 min. This may be attributed to the increase of the photo-generated charges by more photocatalysts. However, compared with that of CNZOS, CNZO (25 mg/l) showed a lower disinfection efficiency of 81.99% after 120 min, within a larger CNZO content. Colony formation was almost completely prevented when bacteria were treated with CNZOS₄, where the numbers of colonies could be seen on LB agar treated with CNZO (Fig. 6). These results confirm that CNZOS₄ exhibited better inhibition ability toward bacteria than CNZO. The result of a control experiment indicated that visible light alone caused no photolysis of bacterial cells. Furthermore, before the photocatalytic treatment, *S. aureus* cells were absorbed onto the surface of CNZOS₄. After photocatalytic processing, the cell membrane wrinkled structure appeared. With the extension of processing time, the bacterial membrane became more and more crimped. The cell membrane damage was due to the ROS generated by CNZOS nanohybrids [22, 31]. After the destruction of the cell membrane by CNZOS₄ treatment, the growth of bacteria was further inhibited. According to the calculation by UV-Vis DRS, as shown in Fig. 7, the transportation of h⁺ and e⁻ at the interface of CNZOS₄ under visible-light irradiation was demonstrated. Based on the above results, the possible infection mechanism of CNZOS₄ is proposed in Fig. 8. This displays that visible-light irradiation of g-C₃N₄ generated conduction band electrons (e⁻) and valence band holes (h⁺), and the embedded ZnO acted as electron traps to facilitate

Fig. 7. Generation, separation, and transportation of h⁺ and e⁻ at the interface of CNZOS₄ under visible-light irradiation.

the separation of photogenerated electron-hole pairs and promoted the interfacial electron transfer process. Furthermore, the CNZOS nanohybrids increased the efficiency of charge carrier separation, extended the range of light absorption, and facilitated the creation of electron-hole pairs [39]. The increased generation of ROS may therefore be the dominant mechanism for CNZOS_x nanohybrids, which have an enhanced antibacterial activity when compared with the CNZO composite. As demonstrated by the reaction discussed above, the CNZOS_x nanohybrids showed very high ROS generation ability (Fig. 9).

Fig. 8. Mechanism of *S. aureus* inactivation in the presence of CNZOS₄ under visible light.

Photocatalytic Disinfection Mechanism

Owing to the oxidative strength difference, the disinfection capability of each ROS between CNZOS₄ and CNZO was investigated through scavenger-quenching experiments. The results are shown in Figs. 9 and 10. This is consistent

with the fact that CNZOS₄ demonstrated a higher disinfection efficiency and proves the effectiveness of stellerite in ROS generation. All these results are consistent with the band structure of CNZOS_x, which favors oxygen reduction to produce ROS (Fig. 7).

Fig. 9. Scavenger-quenching experiments with CNZOS₄.

Colored lines represent the results of disinfection experiments conducted in the presence of scavengers. (A) Photocatalytic disinfection performance with scavenger Fe(II) to quench photogenerated H₂O₂. (B) Photocatalytic disinfection performance with scavenger sodium oxalate to quench photogenerated h⁺. (C) Photocatalytic disinfection performance with scavenger TEMPO to quench photogenerated •O₂⁻. (D) Photocatalytic disinfection performance with scavenger sodium chromate to quench photogenerated e⁻. (E) Photocatalytic disinfection performance with scavenger isopropanol to quench photogenerated OH•.

For the scavenger-quenching results in the CNZOSx system, the trend of ROS-scavenging effect was similar to that of CNZO, but the change in disinfection rate was more obvious. The scavenging effect from high to low in the CNZOSx system was Fe(II)-EDTA (for H₂O₂), sodium

oxalate (for h⁺), TEMPO (for •O₂⁻), Cr (VI) (for e⁻), and isopropanol (for OH•). There was only a 1 Ln inactivation of bacteria after 2 h of visible-light irradiation within Fe(II). The results suggest that H₂O₂ has the strongest effect in inactivating bacteria, and a better electron-hole pair

Fig. 10. Scavenger-quenching experiments with CNZO.

Colored lines represent the results of disinfection experiments conducted in the presence of scavengers. (A) Photocatalytic disinfection performance with scavenger Fe(II) to quench photogenerated H₂O₂. (B) Photocatalytic disinfection performance with scavenger sodium oxalate to quench photogenerated h⁺. (C) Photocatalytic disinfection performance with scavenger TEMPO to quench photogenerated •O₂⁻. (D) Photocatalytic disinfection performance with scavenger sodium chromate to quench photogenerated e⁻. (E) Photocatalytic disinfection performance with scavenger isopropanol to quench photogenerated OH•.

separation was enabled by adding stellerite as a carrier. This result is consistent with the reports that H_2O_2 played a critical role in the inactivation of bacteria [40]. Otherwise, this result suggests that h^+ is also important in the bacterial inactivation. It is possible that quenching of h^+ could promote e^- and related ROS generation so that the overall disinfection rate change was much higher than that in the CNZO system. However, $\text{OH}^\bullet$ did not show much inactivation effect since no slowing down of bacterial inactivation rate was observed. This could be due to the extremely short life-span of $\text{OH}^\bullet$, which is supposed to be quickly quenched in aqueous media before it can cause damage to the bacterial cell [41]. In addition, h^+ -related generation of $\bullet\text{O}_2^-$ and H_2O_2 was of higher concentration than that of CNZO.

In the CNZO system, the scavenging effect from high to low in the visible system according to the reaction rate was Fe(II)-EDTA, TEMPO, Cr(VI), sodium oxalate, and isopropanol. The difference in the CNZO system comparing with the CNZOSx system is that H_2O_2 contributes the most to bacteria disinfection. The scavenging effect of $\bullet\text{O}_2^-$ becomes more unobvious, suggesting the important role of $\bullet\text{O}_2^-$. Owing to disproportionate properties, $\bullet\text{O}_2^-$ in water was very unstable instead of reacting with DMPO. This confirmed the role of stellerite in $\bullet\text{O}_2^-$ generation. Based on the above reactive species research, it is more likely that holes may inactivate bacteria directly and indirectly by H_2O_2 [26, 41].

In summary, for the scavenger-quenching results in the CNZOSx system, the trend of ROS-scavenging effect was different from that of CNZO, and the change in disinfection activity was more obvious. With stellerite as a carrier, the aggregation of CNZO and recombination of free charges were less serious. Rapid separation and transportation of photogenerated electrons–holes enhanced the generation of ROS. The scavenging experiments prove that in both the CNZO and CNZOSx systems, holes may inactivate bacteria directly or indirectly via H_2O_2 , $\bullet\text{O}_2^-$, and $\text{OH}^\bullet$.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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