Synthesis of Carbon Doped TiO₂ Quantum Dots for Photocatalytic Sterilization under the Visible Light Irradiation and the Mechanisms

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Abstract. In this article, the carbon doped TiO₂ (C-TiO₂) quantum dots (QDs) were prepared through the hydrothermal method and calcination. The size of the C-TiO₂ QDs is about 5.7 nm. The doping amount of carbon can be tuned by adjusting the volumes of the carbon source, ethylene glycol added. The carbon atoms are proved to be doped into the interstitial sites of TiO₂ lattice and induce the change of chemical states of Ti 2p and C 1s. The doping of carbon leads to the increasing photocatalytic sterilization of *E. coli* under the visible light irradiation. The survival rate of *E. coli* cells over C-TiO₂ is only 1.5 % after 6 h. The reactive oxygen species (ROS), such as hydroxyl radical and superoxide radical, are considered as the primary factors for the photocatalytic sterilization. Due to oxidative stress of the attack by ROS, the enzyme activity per cells increases for self-protection during the photocatalytic sterilization.

1 Introduction

Waste water from hospitals and food factories usually contains microorganisms, virus, which is threatening the safety of our drinking water [1]. The TiO₂-based photocatalytic technology attracts increasing attention as an alternative promising method in the application of sterilization, due to the low cost, low toxicity, utilization of sunlight, and no secondary pollution [2]. During the process, TiO₂ can generate reactive oxygen species (ROS) under the irradiation of light, which show strong oxidizing ability in the decomposition of organic compounds and microorganisms. However, it only responses to the UV light due to the large band gap (3.20 eV for anatase), which accounts less than 5% among the sunlight [3]. Hence, a lot of attempts including metal element doping, nonmetal element doping, semiconductor modification, noble metal deposition and so forth have been developed to sensitize TiO₂ for the much larger visible fraction [4]. Among the elements for doping, the carbon has received much attraction benefitting from the advantages in narrowing of the band gap and extending the light absorbance of TiO₂ into visible region, avoiding charge recombination, and good thermal stability [5]. Actually, the C-TiO₂ has been widely applied in microbial inactivation [6], water splitting [7], pollutant degradation [8] etc.

The morphological regulation is also beneficial to improve the photocatalytic performance of the semiconductors [9]. Benefiting from the high surface-to-volume ratio, rich surface binding properties, the utilization of hot electrons, and the generation of

multiple charge carriers from single high-energy photons, the semiconductor QDs have been widely used in the photocatalytic field [10-12]. Herein, we report the photocatalyst, C-TiO₂ QDs with enhanced visible-light performance. We characterize the C-TiO₂ QDs and evaluate its photocatalytic sterilization effect against *E. coli* with the irradiation of visible light. The change of enzyme activity of cells during the photocatalytic process has also been investigated.

2 Method

2.1. Synthesis of C-TiO₂

The C-TiO₂ QDs were synthesized by the hydrothermal method with minor modification [13]. In a typical process, 1 mL tetrabutyl titanate was added into 25 mL ethylene glycol/Milli-Q water solution and stirred for 30 min. Then the mixed solution was transferred into the autoclave and kept at 180 °C for 12 h. The products were collected by centrifugation after natural cooling and rinsed thoroughly with Milli-Q water and ethanol. The C-TiO₂ QDs were obtained after drying at 60 °C overnight and calcining at 350 °C for 2 h. Different volumes of ethylene glycol (0, 5 mL, 10 mL, 15 mL) were added into water, and the related samples synthesized were named with TiO₂, CTO-1, CTO-2, and CTO-3, respectively.

2.2 Characterization

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The morphology, crystalline structure and chemical valence of C-TiO₂ QDs were observed by transmission electron microscopy (TEM, FEI Tecnai G2), X-ray diffraction (XRD) on Empyrean X-ray diffractometer using Cu K α radiation between 10° to 80° and X-ray photoelectron spectroscopy (XPS, Perkin-Elmer PHI 5600), respectively. The ROS for the samples was investigated by the Electron spin resonance (ESR, Bruker A200) signals with the parameters as following: center field = 3400 G, frequency = 9.46 GHz, microwave power = 20.02 mW. 5 mL 0.1 M 5, 5-dimethyl-1-pyrroline-N-oxide (DMPO) solution were used as the spin-trap reagent.

2.3 Photocatalytic sterilization

The photocatalytic sterilization performance of the as-prepared C-TiO₂ was evaluated under the simulative visible light for the inactivation of *E. coli*. A 300W xenon lamp with a 400 nm cutoff filter was used as light source. The viable *E. coli* (K13) cells were washing with saline for three times and dispersed in saline. The concentration of the *E. coli* suspension was about 6×10^8 CFU mL⁻¹. In every experiment, 10 mg of the photocatalyst was dispersed into 40 mL *E. coli* suspension. The mixed *E. coli* suspension was stirred in the dark for 30 min before adding light irradiation and 1 mL of the reaction solution was collected every hour, diluted immediately with saline and spread uniformly over nutrient agar plates. The microbial colonies were counted after incubated at 37 °C for 12 h in dark. In the enzyme activity test, 1 mL reaction solution was also collected at given time. The enzyme activities were analyzed by the catalase (CAT) assay kit (A007-1-1, Nanjing Jiancheng Bioengineering Institute) and total superoxide dismutase (SOD) assay kit (A001-1-2, Nanjing Jiancheng Bioengineering Institute) and checked by recording changing of the UV-vis absorption band at 405 nm and 550 nm by UV1100 spectrophotometer (Techcomp).

3 Results and discussion

3.1. Characterization of C-TiO₂ QDs

The C-TiO₂ QDs were synthesized through a simple hydrothermal method and calcination. The tetrabutyl titanate acted as the Ti precursor and the ethylene glycol was used as the carbon source. The doping amount of carbon could be adding different volumes of ethylene glycol. The molar fractions of carbon by adding 5, 10, 15 mL ethylene glycol in the C-TiO₂ QDs were 9.86%, 12.58 %, and 15.63 %, respectively. The treatment of calcination at 350 °C endues the C-TiO₂ QDs better crystallinity and remove the organic impurity.

The morphology and size of C-TiO₂ QDs were analysed through the TEM (Fig. 1a). The C-TiO₂ particles showed the diameter of about 5.7 nm. The nanocrystal structure of C-TiO₂ could be confirmed further from the HRTEM image (Fig. 1b). The clear lattice fringe spacing of 0.351 or 0.350 nm matched well

with the crystallographic planes of TiO₂ (101). It could indicate that the carbon doping has no impact on the lattice of TiO₂.

Fig. 1. TEM (a) and HRTEM (b) images of C-TiO₂.

The crystal structure of the obtained C-TiO₂ QDs with various carbon content had been investigated by the XRD. As shown in Fig. 2a, the diffraction parents of the samples coincided with the anatase phase TiO₂ (JCPDS no. 21-1272). The peaks at 25.4, 37.9, 48.0, 54.3, 62.9, and 70.3 degree could be indexed to the (101), (004), (200), (105), (204), and (220) planes, respectively. No characteristic peak could be detected in the C-TiO₂ QDs samples, which also indicated that the doping of carbon had no impact on the crystalline phase and crystallinity of the TiO₂. In addition, scarcely any other peaks and intense patterns indicate the high purity and well crystallinity of the as-prepared catalysts.

Fig. 2. (a) XRD patterns of TiO₂, CTO-1, CTO-2, and CTO-3; Ti 2p (b), C 1s (c), and O 1s (d) XPS spectra of CTO-3.

Furthermore, XPS results demonstrated the existence of Ti, C, and O in the C-TiO₂. The spectrum of Ti 2p for TiO₂ with the presence of two peaks at 458.6 eV and 464.3 eV were assigned as Ti 2p_{3/2} and Ti 2p_{1/2} spin-orbital splitting photoelectrons in the Ti⁴⁺ chemical state, respectively (Fig. 2b). However, the peaks shifted to 458.3 eV and 464.0 eV with the doping of carbon. The change of the Ti⁴⁺ chemical state indicated that carbon was doped into the interstitial sites of TiO₂ lattice [14]. The peaks for C 1s located at 285.7 and 288.2 eV were assigned to the C-O and O=C-O, while the typical peak at 284.5 eV was attributed to adventitious contamination from the ambient environment (Fig. 2c) [15]. After doping with carbon, the intensity of the peak at 285.7 eV

increased obviously, indicating the formation of C-O bond in C-TiO₂. In the spectrum of O 1s (Fig. 2d), three peaks were observed at the binding energies of 529.4 eV, 529.8 eV and 530.7 eV, which could be attributed to the oxygen in the lattice (Ti-O bond, Ti-O-C bond), and adsorbed oxygen atoms (Ti-OH group), respectively.

3.2 Photocatalytic sterilization

The *E. coli* cells were selected as the target microorganisms to discuss the photocatalytic sterilization performances of C-TiO₂ under the visible-light irradiation. The experiments on photocatalytic sterilization of *E. coli* by C-TiO₂ QDs with different content of carbon were comparatively performed (Fig. 3). The sterilization of *E. coli* by photolysis without the catalyst could be neglected. Under the simulated visible light irradiation for 6 h, the survival rate of *E. coli* over CTO-3 was only 1.5 %, while the survival rates over CTO-2, CTO-1, and TiO₂ were about 46.9 %, 71.5%, and 82.9%, respectively. The corresponding kinetic constants of the sterilization of *E. coli* over CTO-3, CTO-2, CTO-1, and TiO₂ were 0.314, 0.045, 0.024, and 0.014 h⁻¹, respectively. It was clear that CTO-3 exhibited the highest photocatalytic activity for inactivation of *E. coli*. The prominent performance of CTO-3 could be attributed to the improvement of the visible light harvesting. Thanks to the increasing concentration of carbon dopant, the absorption region of the catalysts has been obviously extended to the visible light. Besides, the conductivity of TiO₂ can be improved after doping of carbon, which would promote the transfer of the photogenerated charge to the surface. Taking these advantages, it is not surprising that the C-TiO₂ exhibit improved photocatalytic sterilization effect than that of TiO₂ and the activity continues improving by increasing the amount of carbon from 9.86% to 15.63 %.

ROS generation is considered as the most common factor for the photocatalytic sterilization. The ROS for the sterilization mainly includes superoxide radical ($\bullet\text{O}_2^-$), hydroxyl radical ($\bullet\text{OH}$), and peroxide (H₂O₂), which can lead to a series of oxidative processes and ultimately cause cell damage (eg., lipid preoxidation, protein oxidation, and DNA damage) and even cell death. However, the ROS over TiO₂ catalysts is generated mainly under UV illumination. The doping of carbon into TiO₂ extends of the absorption range from UV into the visible region. The ROS generation of TiO₂, CTO-1, CTO-2, CTO-3 during the photocatalysis under the visible light irradiation has been investigated by the ESR spectra, as shown in Fig. 4. It can be observed that there are bare signals of $\bullet\text{OH}$ or $\bullet\text{O}_2^-$ generated over the TiO₂. Therefore it is not surprising that TiO₂ shows very poor antibacterial activity under visible light irradiation. As a comparison, the peaks of the signals for $\bullet\text{OH}$ or $\bullet\text{O}_2^-$ rised after doping carbon and the intensities of the signals increased with the increasing carbon doping amount. The results of ESR also indicated the highest photocatalytic inactivation for of *E. coli* over CTO-3, which was consistent with the previous photocatalytic experiments.

Fig. 3. Inactivation of *E. coli* by photocatalysis with TiO₂, CTO-1, CTO-2, CTO-3 under visible light irradiation (a) and the corresponding kinetic linear fitting (b).

Fig. 4. ESR spectra for different photocatalysts with a) DMPO (a) and DEPMPO (b) spin trap.

ROS can cause a series of cell damage, and lead to the exudation of enzymes in the cell for self-protection while being attacked. Under the stress of ROS, the response of *E. coli* could be detected by checking the change of enzyme activity during the inactivation. Figure 5 indicated the enzyme activity of CAT and SOD during the inactivation of *E. coli* with or without CTO-3. It could be seen that the enzyme activities of CAT and SOD increased firstly, reaching about 0.7 U mL⁻¹ and 3.7 U mL⁻¹, respectively, and then decreased due to the inactivation of *E. coli* by the photooxidation of CTO-3. Due to the attack of ROS, the cells would produce CAT and SOD to protect themselves. Although the continuing increasing oxidative stress caused cells to secrete more enzymes, a large number of cell death led to the reduction of enzyme activity. In addition, during the experiment, the enzyme activities for the inactivation with CTO-3 was a slightly lower than that without the catalyst. Considering the concentration of viable cells in the solution, it could be believed that the CAT or SOD produced per cell during the photocatalytic process were much higher than that of photolytic reaction. It can also

confirm the irreplaceable roles of the ROS during the photocatalytic sterilization.

Fig. 5. Enzymic activity of catalase (a) and SOD (b) with or without CTO-3 during the inactivation of *E. coli*.

Based on the references [16, 17] and the above results, the photocatalytic sterilization process and mechanism can be proposed as following. Firstly, the photogenerated electron-hole pairs generated from the C-TiO₂ under the irradiation of visible light (1). The photogenerated electrons transferred to the surface of catalysts and reacted with the adsorbed oxygen to yield •O₂⁻ (2), whereas the holes accumulated in the valence band could interact with surface-bound H₂O or OH⁻ to produce the •OH radical species (3, 4). The generated •OH could combine to produce H₂O₂ (5), and further react with •O₂⁻ to generate •OH (6). The ROS, such as •OH, •O₂⁻ and H₂O₂, could oxidize and decompose the cell membrane and wall and cause lipid preoxidation, DNA damage, and protein oxidation, which led to the cell death (7). The relevant reactions over the C-TiO₂ QDs for photocatalytic sterilization can be expressed as follows:

4 Conclusion

In summary, titanium dioxide-based photocatalysts with enhanced visible light activity have been developed for sterilization by doping carbon. The C-TiO₂ QDs are synthesized through the hydrothermal method and calcination. The carbon atoms are doped into the interstitial sites of TiO₂ lattice and lead the change of chemical states of Ti 2p and C 1s. The doping of carbon increases the photocatalytic sterilization of *E. coli* under the visible light irradiation and the increasing amount of carbon doping can improve the photocatalytic sterilization. The ROS, such as hydroxyl radical and superoxide radical, are the primary factors for the photocatalytic inactivation of *E. coli*. Due to oxidative stress of the attack by ROS, the enzyme activity per cells increases for self-protection during the photocatalytic sterilization.

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