Synthesis of Carbon Doped TiO$_2$ Quantum Dots for Photocatalytic Sterilization under the Visible Light Irradiation and the Mechanisms

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Abstract. In this article, the carbon doped TiO$_2$ (C-TiO$_2$) quantum dots (QDs) were prepared through the hydrothermal method and calcination. The size of the C-TiO$_2$ 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 TiO$_2$ 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-TiO$_2$ 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 TiO$_2$-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, TiO$_2$ 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 responds 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 TiO$_2$ for the much larger visible fraction [4]. Among the elements for doping, the carbon has received much attraction benefiting from the advantages in narrowing of the band gap and extending the light absorbance of TiO$_2$ into visible region, avoiding charge recombination, and good thermal stability [5]. Actually, the C-TiO$_2$ 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 genetation 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-TiO$_2$ QDs with enhanced visible-light performance. We characterize the C-TiO$_2$ 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-TiO$_2$

The C-TiO$_2$ 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-TiO$_2$ 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 TiO$_2$, 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 Ka 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-pirroline-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 × 10⁸ 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 total catalase (C) endues the C-TiO₂ QDs better crystallinity and remove the organic impurity.

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 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.

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.

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 2p½ and Ti 2p½ 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 (•O2⁻), hydroxyl radical (•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 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 been observed that there are bare signals of •OH or •O2⁻ 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 •OH or •O2⁻ raised 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).](image)

![Fig. 4. ESR spectra for different photocatalysts with a) DMPO (a) and DEPMPO (b) spin trap.](image)
confirm the irreplaceable roles of the ROS during the photocatalytic sterilization.

![Image](image-url)

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-TiO$_2$ under the irradiation of visible light (1). The photogenerated electrons transferred to the surface of catalysts and reacted with the adsorbed oxygen to yield •O$_2^-$ (2), whereas the holes accumulated in the valence band could interact with surface-bound H$_2$O or OH$^-$ to produce the •OH radical species (3, 4). The generated •OH could combine to produce H$_2$O$_2$ (5), and further react with •O$_2^-$ to generate •OH (6). The ROS, such as •OH, •O$_2^-$ and H$_2$O$_2$, could oxidize and decompose the cell membrane and wall and cause lipid preoxidation, DNA damage, and protein oxidation, which leaded to the cell death (7). The relevant reactions over the C-TiO$_2$ QDs for photocatalytic sterilization can be expressed as follows:

$$\text{C-TiO}_2 + \text{hv} \rightarrow \text{C-TiO}_2 (\text{e}^-_{cb} + \text{h}^+_{vb})$$ (1)

$$\text{O}_2 + \text{e}^-_{cb} \rightarrow \text{•O}_2^-$$ (2)

$$\text{H}_2\text{O} + \text{h}^+_{vb} \rightarrow \text{•OH} + \text{H}^+$$ (3)

$$\text{OH}^- + \text{h}^+_{vb} \rightarrow \text{•OH}$$ (4)

$$\text{•OH} + \text{OH}^{-} \rightarrow \text{H}_2\text{O}_2$$ (5)

$$\text{•O}_2^- + \text{H}_2\text{O} \rightarrow \text{•OH} + \text{OH}^- + \text{O}_2$$ (6)

$$\text{•OH}, \text{•O}_2^-, \text{H}_2\text{O}_2 + \text{E. coli} \rightarrow \text{Inactivated E. coli}$$ (7)

4 Conclusion

In summary, titanium dioxide-based photocatalysts with enhanced visible light activity have been developed for sterilization by doping carbon. The C-TiO$_2$ QDs are synthesized through the hydrothermal method and calcination. The carbon atoms are doped into the interstitial sites of TiO$_2$ 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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