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Metal-free inactivation of *E. coli* O157:H7 by fullerene/C_3N_4 hybrid under visible light irradiation

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ABSTRACT

Interest has grown in developing safe and high-performance photocatalysts based on metal-free materials for disinfection of bacterial pathogens under visible light irradiation. In this paper, the C_{60}/C_3N_4 and C_{70}/C_3N_4 hybrids were synthesized by a hydrothermal method, and characterized by X-ray diffraction (XRD), UV-vis diffuse reflection spectroscopy (UV–vis DRS), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and high revolution transmission electron microscope (HRTEM). The performance of photocatalytic disinfection was investigated by the inactivation of *Escherichia coli* O157:H7. Both C_{60}/C_3N_4 and C_{70}/C_3N_4 hybrids showed similar crystalline structure and morphology with C_3N_4; however, the two composites exhibited stronger bacterial inactivation than C_3N_4. In particular, C_{70}/C_3N_4 showed the highest bactericidal efficiency and was detrimental to all *E. coli* O157:H7 in 4 h irradiation. Compared to C_3N_4, the enhancement of photocatalytic activity of composites could be attributed to the effective transfer of the photoinduced electrons under visible light irradiation. Owing to the excellent performance of fullerenes (C_{60}, C_{70})/C_3N_4 composites, a visible light response and environmental friendly photocatalysts for disinfection were achieved.

1. Introduction

The disinfection of pathogenic microorganisms in drinking water is crucial for the human health, as millions of deaths and disease cases are caused by water contaminated by pathogens in the world every year (Burch and Thomas, 1998; Clasen and Edmondson, 2006). Traditional disinfection strategies such as chlorination and ozonation are highly effective for inactivation; however, they usually produce harmful by-products with carcinogenic and mutagenic potential (Muellner et al., 2007). Therefore, development of practical and safe technologies for drinking water purification has become an urgent demand (Chong et al., 2010; Dalrymple et al., 2010; Helali et al., 2014).

UV irradiation disinfection does not have the problems associated with chlorination and ozonation, but UV itself is hazardous and energy intensive. UV driven photocatalytic inorganic nanomaterials, such as titania, can only be activated by wavelengths in the near UV region ($\lambda <$ 400 nm) and cannot efficiently utilize the major spectrum of sunlight to photocatalytically destroy the microorganisms (Zhang et al., 2013). Hence, visible light driven (VLD) photocatalytic technology for microbial disinfection shows promising prospects for its unique advantages including low cost, chemical stability, non-toxicity, and effectiveness. Until now, several types of VLD photocatalysts have been applied to the photocatalytic destruction of microorganisms under visible light (VL) irradiation. For example, Liu et al. (2012) revealed that the Ag/TiO_2 nanofiber membrane achieved 99.9% *E. coli* inactivation under solar irradiation within 30 min. Elahifard et al. (2007) demonstrated that the prepared Ag/AgBr/TiO_2-covered apatite showed a high binding capability and photocatalytic disinfection activity to *E. coli* under VL irradiation, 1×10^7 cfu mL^{-1} of *E. coli* could be killed completely by this composite within 3 h. Wang et al. (2013a) illustrated that total inactivation of about 7 log of *E. coli* K-12 cells was achieved after 3 h of irradiation in the presence of 100 mg L^{-1} $CdIn_2S_4$. Nevertheless, the use of these photocatalysts is restricted because of the ever-present release of heavy metal ions. For instance, the leakage of Ag^+ from Ag/AgBr/TiO_2 and AgBr–Ag–Bi_2WO_6 were 0.27 and 0.55 mg L^{-1}, respectively, (Zhang et al., 2010; Hou et al., 2012) which were beyond the US EPA (Environmental Protection Agency) drinking water standard of 0.1 mg L^{-1}. The elution of Cd^{2+} from $CdIn_2S_4$ was as high as 0.2 mg L^{-1}, which was 40 times than US EPA drinking water standard (Wang et al., 2013a). Therefore, developing safe and high-performance photocatalysts based on metal-free materials for disinfection under VL irradiation is desired.

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Carbon nitride (C_3N_4) as a metal-free photocatalyst has received a large interest as the most promising candidate for VL-responsive photocatalysts. Even in this early stage, C_3N_4 together with their modifications have been widely used in relevant fields of chemistry (Thomas et al., 2008; Wang et al., 2009, 2010; Zhu et al., 2010). Most of the studies about C_3N_4 usually focused on potential applications in energy conversion (Ito et al., 2008; Vinu, 2008), hydrogen (Bai et al., 2001; Yang et al., 2009; Li et al., 2010a) and carbon dioxide storage (Haque et al., 2010), degradation of pollutants (Zhou et al., 2002), solar cells (Lee et al., 2008b; Noto and Negro, 2010; Zhang and Antonietti, 2010), humidity and gas sensors (Lee, 2008a; Su et al., 2010). However, studies on photocatalytic inactivation of microorganisms by C_3N_4 are very limited. Very recently, Wang et al. (2013b) synthesized the graphene and graphitic-C_3N_4 (g-C_3N_4) nanosheets co-wrapped elemental*α*-sulfur as a metal-free photocatalyst for bacterial inactivation. This composite exhibited high photocatalytic bacterial inactivation activities, and bacterial cells were completely killed after 4 h VL irradiation. Additionally, it has been reported that fullerenes (C_{60}, C_{70}) represent a well known allotrope of carbon with unique electronic properties, which can act as an excellent electron acceptor (Bhattacharya et al., 2004; Cremer et al., 2006) and efficiently cause a rapid photoinduced charge separation and a relatively slow charge recombination (Yu et al., 2011). The combination of fullerene to C_3N_4 may further enhance the photocatalytic disinfection activity which has never been reported.

In this study, novel metal-free photocatalysts were prepared by cowrapping fullerene (C_{60}, C_{70}) with C_3N_4 using a hydrothermal process. The photocatalytic inactivation activity of the composites was investigated under VL illumination using *E. coli* O157:H7, which is responsible for many outbreaks of gastrointestinal illness and hemolytic uremic syndrome worldwide (Boyce et al., 1995). In addition, possible photocatalytic inactivation mechanisms were proposed based on the obtained experimental results.

2. Materials and methods

2.1. Preparation and characterizations of materials

C_3N_4 were synthesized by thermal treatment using urea as the starting material (Chai et al., 2014). Briefly, urea was heated at 600 °C for 2 h in static air with a ramping rate of 10 °C min^{-1}. The resultant yellow agglomerates were grinded into fine powders for further use.

Fullerenes (C_{60}, C_{70})/C_3N_4 composites were prepared in a hydrothermal process according to the reference (Chai et al., 2014). The details were as follows: an appropriate amount of C_{60} or C_{70}, 35 mL of concentrated nitric acid and 5 mL of deionized water were added into a 100 mL of beaker with magnetic stirring. After heating for 2 h and stewing for a while, the reaction mixture was washed to be neutral with ultrapure water. The prepared C_3N_4 powder was added to the above solution and sonicated for 20 min, the reaction mixture was then transferred into a 50 mL of hydrothermal synthesis reactor and underwent hydrothermal treatment at 160 °C for 4 h. After being cooled to ambient temperature, the precipitation was collected by centrifugation, then dryed and grinded to obtain the C_{60} or C_{70} hybridized C_3N_4 samples. Finally, the product was calcined in a muffle furnace at 400 °C for 5 h, the received bulk fullerene/C_3N_4 was milled into powders. The mass ratio of fullerene to C_3N_4 in the composite is 1:50.

X-ray diffraction (XRD) patterns were recorded with a Rigaku Smart Lab X-ray diffractometer using Cu K*α*1 irradiation (λ=1.5406 Å). The accelerating voltage and applied current were 40 kV and 40 mA, respectively. UV–visible diffuse reflectance spectra (UV–vis DRS) of the powders were obtained for the dry-pressed disk samples using a Varian Cary 500 UV–vis spectrophotometer equipped with a labsphere diffuse reflectance accessory. $BaSO_4$ was utilized as a reflectance standard in the UV–vis DRS experiment. The general morphologies of the products were characterized by scanning electron microscopy equipped with an energy-dispersive X-ray (EDX) spectrometer. The HRTEM measurement was conducted using a JEOL JEM 2100F microscope working at 200 kV. The functional groups of composites were characterized by a Fourier transform infrared spectrometer (FT-IR, Nicolet 670).

2.2. Culture and collection of E. coli O157:H7

E. coli O157:H7 selected in this study was obtained from State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University. Cells were inoculated overnight in Luria-Bertani (LB) at 37 °C and 200 rpm, followed by culturing in LB for 3.5 h at 37 °C to reach mid-exponential growth phase. After reaching this growth stage, the culture was harvested by centrifugation at 4 °C and 3700 g for 15 min to separate the cells from the growth media. During the harvesting process, the cells were rinsed two additional times using 1 mM KCl to remove any remaining growth media residue from the cells. The cells were then re-suspended in 5 mL of electrolyte with the same solution chemistry as subsequent experiments.

2.3. Photocatalytic bacterial inactivation

The VLD photocatalytic inactivation of *E. coli* O157:H7 was conducted using a 300 W xenon lamp with a UV cutoff filter (λ > 420 nm) as light source. The VL intensity was measured by a light meter (LI-COR, USA) and the light intensity for the experiments was fixed at 193 mW cm^{-2}. The light intensity was calculated by the sum of light intensity which above 420 nm. All glass apparatuses used in the experiments were autoclaved at 121 °C for 20 min to ensure sterility. In a typical run, an appropriate amount of photocatalyst and a certain concentration of bacterial culture were dispersed in a 100 mL reaction vessel, then 1 mM KCl was added into the vessel reaching a total volume of 30 mL (the final bacterial density was 0.5 mg mL^{-1}). The reaction temperature was maintained at 25 °C using condensate water and the reaction mixture was stirred with a magnetic stirrer throughout the experiment. Before irradiation, the suspensions were magnetically stirred in dark for 10 min to ensure the establishment of an adsorption/desorption equilibrium between the photocatalyst and bacterial cells. At 20 min interval time, 1 mL of the sample were collected and serially diluted with sterilized saline solution, and then 0.1 mL of the diluted sample was immediately spread on MacConkey Agar plates and incubated at 37 °C for 24 h to determine the number of viable cells (in cfu). All the photocatalytic bacterial inactivation experiments were performed in triplicates. A bacterial control without photocatalyst was also set-up, while another photocatalyst control was kept in the dark. The time-dependent survival data of bacteria were fitted into the Chick-Watson model for a batch process: $\ln(N/N_0) = -kt$, where N_0 represents the cell concentrations of *E. coli* O157:H7 before irradiation and N represents the cell concentrations after being irradiated, k is the pseudo-first-order kinetic constant (Sapkota et al., 2011). The experimental setup was shown in Fig. S1 (Supplementary material). The light spectrum of the Xenon Lamp was supplied in Fig. S2.

3. Results and discussion

3.1. Hybrid structure and morphology

XRD was employed for analyzing the crystalline phase of different samples. As shown in Fig. 1, the C_3N_4 displays its characteristic pattern dominated by the interlayer-stacking (002) reflection. Additionally, minor reflections are also observed among which the (100) interlayer reflection is the most intense (Yan et al., 2009; Dong et al., 2011). Compared with bare C_3N_4, the diffraction peak position and shape of C_3N_4 composites with C_{60} and C_{70} did not change, suggesting that the introduction of C_{60} or C_{70} had no influence on the crystalline structure