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Effect of aqueous media on the copper-ion-mediated phototoxicity of CuO nanoparticles toward green fluorescent protein-expressing *Escherichia coli*

Enxiang Shang, Yang Li, Junfeng Niu*, Huiyuan Guo, Yijing Zhou, Han Liu, Xinqi Zhang

State Key Laboratory of Water Environment Simulation, School of Environment, Beijing Normal University, Beijing 100875, People's Republic of China

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ABSTRACT

Quantitative comparison of different aqueous media on the phototoxicity of copper oxide nanoparticles (CuO NPs) is crucial for understanding their ecological effects. In this study, the phototoxicity of CuO NPs toward the green fluorescent protein-expressing *Escherichia coli* (GFP-*E. coli*) under UV irradiation (365 nm) was investigated in Luria-Bertani medium (LB), NaCl solution, deionized water (DI) and phosphate-buffered saline (PBS). The phototoxicity of CuO NPs toward GFP-*E. coli* decreased in the order of DI > NaCl > PBS > LB because of different released concentrations of Cu^{2+}. The 3 h released Cu^{2+} concentrations by 10 mg/L CuO NPs in DI water, NaCl solution, LB medium, and PBS were 1946.3 ± 75.6, 1242.5 ± 47.6, 1023.4 ± 41.2, and 1162.1 ± 41.9 μg/L, respectively. Transmission electron microscope and laser scanning confocal microscope images of *E. coli* exposed to CuO NPs demonstrated that the released Cu^{2+} resulted in fragmentation of bacterial cell walls, leakage of intracellular components, and finally death of bacteria in four media after UV light irradiation. In each medium, the bacterial mortality rate logarithmically increased with the releasing concentrations of Cu^{2+} by CuO NPs ($R^2 > 0.90$) exposed to 3 h UV light. This study highlights the importance of taking into consideration of water chemistry when the phototoxicity of CuO NPs is assessed in nanotoxicity research.

1. Introduction

Metal oxide nanoparticles (NPs) have been widely applied in photoactive antimicrobials due to their unique photocatalytic properties (Nel et al., 2006; Li et al., 2012b, 2014c). Copper oxide nanoparticles (CuO NPs) are frequently applied as antimicrobial agents against both Gram-positive and Gram-negative bacteria such as *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (Malka et al., 2013; Martineau et al., 2014). CuO NPs will be eventually released into aqueous environment intentionally or accidently during their production, transportation, application, and disposal (Hu et al., 2014; Rossetto et al., 2014). Therefore, it is crucial to investigate the transformation and photoinduced toxicity of CuO NPs to bacteria in aqueous environment.

E. coli are commonly used as a good biological model to assess the toxicity of NPs because of their well-characterized physiological properties and rapid toxicity screening (Brayner et al., 2006; Baek and An, 2011). Although many previous studies have investigated the toxicity of CuO NPs toward *E. coli* (Choi et al., 2010; Baek and An, 2011; Dasari et al., 2013), differences in the experimental conditions largely hampered the quantitative comparisons among different studies on the toxicity of CuO NPs. For example, CuO NPs dispersed in physiological saline had a median lethal concentration (LC_{50}) of 0.16 mg/L (Dasari et al., 2013), whereas CuO NPs suspended in Mueller-Hinton agar medium or Luria-Bertani medium (LB) was less toxic to *E. coli* with LC_{50} values of 2387.2 and 28.6 mg/L, respectively (Baek and An, 2011; Toolabi et al., 2013). The different LC_{50} values of CuO NPs toward *E. coli* were primarily attributed to the dissimilarity, complexity, and variability of the water chemistry (Dasari et al., 2013; Toolabi et al., 2013). The physicochemical properties of aqueous media (such as pH, ionic strength, and organic matter) significantly affected the aggregation, dissolution and bioavailability of CuO NPs (Käkinen et al., 2011; Dasari et al., 2013; Toolabi et al., 2013). Therefore, it is of great importance to investigate the effects of aqueous media on the toxicity of CuO NPs toward *E. coli*.

Light irradiation is another key environmental factor affecting the toxicity of CuO NPs toward *E. coli* (Li et al., 2012b; Dasari et al., 2013), as CuO NPs are photosensitive materials (Li et al., 2012b, 2014a). When illuminated by light with photoenergy greater than

* Corresponding author. Fax: +86 10 5880 7612.
E-mail address: junfengn@bnu.edu.cn (J. Niu).

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the band gap of CuO NPs, their electrons (e^-) are promoted across the band gap to the conduction band with high reducing power, which creates a hole (h^+) in the valence band with high oxidizing power (Xu and Schoonen, 2000; Huang et al., 2009; Li et al., 2012b). The holes induced the photocorrosion of CuO NPs, and consequently enhanced Cu^{2+} concentrations released by NPs (Huang et al., 2009; Li et al., 2012b). It has also been demonstrated that UV or sunlight could significantly enhance the toxicity of CuO NPs toward *E. coli* primarily due to higher concentrations of Cu^{2+} released by CuO NPs exposed to light than that in the dark (Li et al., 2012b; Dasari et al., 2013). However, how aqueous media affect the concentrations of Cu^{2+} released by CuO NPs and their phototoxicity toward *E. coli* remains unclear. In addition, the quantitative relationships between the concentrations of released Cu^{2+} and the phototoxicity of CuO NPs have not been established.

One of the hindrances in studying the NPs-bacterium interaction is lack of a suitable probe for the characterization of the morphological change of bacterium (Brayner et al., 2006; Jiang et al., 2009). Green fluorescent proteins (GFPs) exhibit intrinsic fluorescence and have been expressed in bacteria to facilitate the rapid monitoring of the cause-effect phenomena of the NPs-bacterium system by spectroscopic or microscopic techniques (Gogoi et al., 2006; Choi et al., 2010). Many previous works have constructed GFP-expressing *E. coli* to assess the toxicity of TiO_2 and silver nanoparticles by the change of bacterial fluorescent intensity and morphology (Choi et al., 2010; Jiang et al., 2011). Thus the recombinant *E. coli* containing luminescence markers provide an efficient and visual tool for ecotoxicological research to investigate the phototoxicity of NPs, study the interactions between CuO NPs and bacterium, and characterize the morphological change of *E. coli*.

In this study, the effect of aqueous medium on the phototoxicity of CuO NPs toward GFP-expressing *E. coli* under UV-365 light irradiation was systematically assessed. Four types of aqueous media were chosen to conduct the toxicity tests, including LB medium, 0.85% NaCl solution, deionized (DI) water, and phosphate-buffered saline (PBS) as they were mostly applied in researching the toxicity of NPs. Transmission electron microscope (TEM) and laser confocal scanning microscopy (LCSM) were used to characterize the recombinant bacteria. Furthermore, the released concentrations of Cu^{2+} by CuO NPs were measured and correlated with their phototoxicity toward *E. coli* under UV-365 light irradiation in each medium.

2. Materials and methods

2.1. Nanoparticle characterization

The homogeneous stock suspension of 1 g/L CuO NPs was prepared by dispersing the solid powders of CuO NPs into DI water followed by agitating using a sonicator (KQ 3200DB, Kunshan, Jiangsu, China) at 100 W and 20 kHz for 15 min. The suspension was used immediately after preparation without storage. The average hydrodynamic diameters (Z-Ave), particle size distributions (PSDs), and zeta potentials of CuO NPs at a concentration of 5 mg/L in four media were determined by dynamic light scattering (DLS) on a Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, UK). One mL of diluted CuO suspension was put in a standard macro-cuvette (1 cm in pass length). The temperature was maintained at room temperature (25 ± 1) °C, and the scattering angle was 173°. Four types of media used for DLS measurements were verified to be free of NPs. TEM (Philips EM420, Eindhoven, Netherlands) was used to characterize the morphology of CuO dispersed in different media at a concentration of 5 mg/L. Five μL of CuO NP suspension was placed on the surface of carbon grids, followed by solvent evaporation at room temperature for at least 4 h. The microscope was operated in the bright field mode at an acceleration voltage of 30 kV.

2.2. Photochemical experiments

Chemicals used in this study are provided in Section S1 of Supporting information (SI). The setup described in our previous studies was used to carry out the photochemical experiments (Li et al., 2012a, 2013), including assessment of toxicity, characterization of bacterial morphology, and measurement of dissolved ions and reactive oxygen species (ROS). In each experiment, 100 mL of reaction suspension was poured into watch glass and placed under a 4 W ultraviolet lamp (UVGL-21, UVP, San Gabriel, CA, USA) with a wavelength of 365 nm (UV-365), which was the primary wavelength of UV light irradiation that reached the earth's surface. The mixed suspensions were continuously stirred by magnetic stirring apparatus (78HW-1, Shanghai, China) to avoid the settlement of CuO NPs. The light intensity in the surface of the reaction suspension was 0.6 mW cm^{-2}.

2.3. Assessment of toxicity

The recombinant bacteria were constructed, cultured, and harvested according to previous report (Jiang et al., 2011). For the toxicity assays, the bacteria were mixed with CuO NPs at different concentrations (0.2, 0.5, 1.0, 10 and 100 mg/L). The concentrations of the bacterial cell were approximately 10^8 (colony-forming units) CFU/mL. Toxicity experiments of CuO NPs toward the bacteria were carried out in DI water, 0.85% NaCl solution, LB medium (10 g/L NaCl, 10 g/L tryptone, 5 g/L yeast extract, and 100 mg/L kanamycin at pH 7.0), and 5 mM PBS at pH 7.0. After UV light irradiation of 3 h, the mixed suspensions were collected to determine the bacterial viability using the fluorescence intensity method and the traditional plate count method (Jiang et al., 2011). The fluorescence intensities of the bacteria were measured by Multimode Microplate Readers (Infinite M200, Tecan, USA) with the excitation and emission wavelengths at 488 and 520 nm, respectively. The bacterial mortality rate was presented as the percentage of surviving bacteria, which was dimensionless and calculated by dividing the number of colonies on the sample plate (N_t) by the number of colonies on a control plate (N_0) (no particle exposure) incubated under the same conditions. The control experiments were also carried out in the dark.

2.4. Measurement of ion release

For the measurement of dissolved ions, aqueous suspensions of CuO NPs without bacteria at different initial concentrations (0.2, 0.5, 1, 10, and 100 mg/L) were exposed to UV-365 light. After light exposure for 3 h, 4 mL of CuO NPs suspension was collected and filtered by an centrifugal ultrafilter (Amicon Ultra-4, Millipore, USA) containing porous cellulose membranes with a nominal pore size of 1–2 nm to remove NPs from suspension as reported previously (Li et al., 2012b, 2015). The sorption of Cu ions on the filters in these media was negligible. Copper complexes formed by Cu ions bounded to proteins and biomolecules in LB medium were also negligible. The filtrate was collected and mixed with 2 mL of trace-metal grade HNO_3. The amount of Cu^{2+} released from CuO NPs suspensions was determined by inductively coupled plasma-mass spectrometry (Agilent 7700, Agilent Technologies, Santa Clara, USA). Control experiments were performed in the dark to measure the background dissolution concentrations from aqueous suspensions of CuO NPs.