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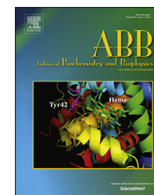

Antibacterial properties and mechanism of graphene oxide-silver nanocomposites as bactericidal agents for water disinfection☆

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ABSTRACT

Providing clean and affordable drinking water without harmful disinfection byproducts generated by conventional chemical disinfectants gives rise to the need for technological innovation. Nanotechnology has great potential in purifying water and wastewater treatment. A graphene oxide-silver (GO-Ag) nanocomposite with excellent antibacterial activity was prepared and characterized by transmission electron microscope and X-ray photoelectron spectroscopy. The tests were carried out using *Escherichia coli* and *Staphylococcus aureus* as model strains of Gram-negative and Gram-positive bacteria, respectively. The effect of bactericide dosage and pH on antibacterial activity of GO-Ag was examined. Morphological observation of bacterial cells by scanning electron microscope showed that GO-Ag was much more destructive to cell membrane of *Escherichia coli* than that of *Staphylococcus aureus*. Experiments were carried out using catalase, superoxide dismutase and sodium thioglycollate to investigate the formation of reactive oxygen species and free silver ions in the bactericidal process. The activity of intracellular antioxidant enzymes was measured to investigate the potential role of oxidative stress. According to the consequence, synergetic mechanism including destruction of cell membranes and oxidative stress accounted for the antibacterial activity of GO-Ag nanocomposites. All the results suggested that GO-Ag nanocomposites displayed a good potential for application in water disinfection.

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1. Introduction

Disinfection of pathogenic microorganisms is an essential process of supplying safe potable water from public water system. Chlorine [1], chlorine dioxide [2], ozone [3], and chloramines [4] are the most common disinfectants in use today. Although these disinfectants can effectively kill microbial pathogens, they can also oxide anthropogenic contaminants, organic matter naturally present in source waters due to their strong oxidizing property, which leads to the formation of disinfection by-products (DBPs) [5], such as trihalomethanes (THMs) [6], haloacetic acids (HAAs) [7], bromate [8] and chlorite [9], the well-known teratogens and carcinogens. Therefore, the demand of developing new generations of antimicrobial agents for effectively killing pathogenic bacteria in drinking water is becoming crucial.

Silver and silver compounds exhibit a strong antibacterial activity to a wide range of microorganisms, including *Pseudomonas aeruginosa*, *Escherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)* and *Candida albicans* [10,11]. Nano-silver is a relatively new and different type of silver with different chemical and physical properties. Silver nanoparticles (AgNPs) show much better antibacterial activity compared with conventional silver-based bactericide [12]. However, the mechanism of antibacterial effect of AgNPs remains not fully understood.

Graphene oxide (GO), a monolayer of carbon atoms that form dense honeycomb structures containing hydroxyl and epoxide functional groups on the two sides and carboxylic groups at the edges, is often used to load metal nanoparticles as these nanoparticles tend to aggregate to minimize their surface energy in the process of preparation [13]. Additionally, it has been proved that GO

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exhibit antibacterial activity against many bacterial species [14,15]. Hence, the introduction of AgNPs onto GO can not only solve the problem of aggregation and stability of AgNPs, but also combine the bactericidal effect of GO and AgNPs. Although many researchers has reported Ag loaded on GO sheets for laboratory and medical devices disinfection [16], purification of fuel and non-polar solvents [17], and food packaging [18], the bactericidal process were both conducted in a static state with agar diffusion method and evaluated by the inhibition zone in these studies. In our tests, the interaction between bactericide and bacteria was carried out in aqueous solution with moderate stirring. Recently, many nanomaterials, such as AgNPs@chitosan-TiO_2 [19], palladium incorporated ZnO [20], silica-silver nanocomposites [21], and iron oxide magnetic nanoparticles [22], were explored as disinfectants for drinking water treatment, but, to our knowledge, the use of silver nanoparticles loaded on GO sheets as bactericides for water disinfection was rare. Compared with AgNPs@chitosan-TiO_2 and palladium incorporated ZnO, the disinfection using silver nanoparticles loaded on GO sheets did not need optical radiation for photocatalysis. And the distribution of silver nanoparticles were more homogeneous with GO than silica-silver nanocomposites and iron oxide magnetic nanoparticles.

In this work, we synthesized graphene oxide-silver (GO-Ag) nanocomposites and used it as bactericide for drinking water disinfection. Gram-positive bacteria, *S. aureus*, and Gram-negative bacteria, *E. coli*, were selected as models to investigate the antimicrobial properties of the composites towards different species of bacteria. Antibacterial activity under different conditions including different bactericide dosage and pH was investigated. The antibacterial mechanism of GO-Ag was also explored by morphological observation, bactericidal species and bactericidal process analysis.

2. Materials and methods

2.1. Materials

Graphite powders were obtained from Jin-Shan-Ting New Chemical Factory (Shanghai, China). Sodium nitrate ($NaNO_3$), sulfuric acid (H_2SO_4, 98%), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2, 30%), and absolute ethanol (CH_3CH_2OH) were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Silver nitrate ($AgNO_3$) was supplied by Beijing Chemical Factory (Beijing, China). Ammonium formate ($HCOONH_4$) was obtained from Kermel Chemical Reagent Company (Tianjin, China). Catalase (CAT), superoxide dismutase (SOD) and sodium thioglycollate (NATG) were purchased from Xiya Chemical Industry Company (Shandong, China). All chemicals in this study were analytical grade and were used as received without any further purification.

The strains employed in this work were the Gram-negative bacterium *E. coli* (ATCC 25922) and the Gram-positive bacterium *S. aureus* (ATCC 6538), which were purchased from the China Center for Type Culture Collection (Wuhan, China).

2.2. Preparation of GO suspension

GO was prepared from powder graphite by adopting modified Hummer and Offeman method [23,24]. Concretely, 1 g graphite powders, 0.5 g $NaNO_3$ and 23 mL concentrated H_2SO_4 were added into a 1 L beaker flask. Under ice bath and stirring condition, 3 g $KMnO_4$ was added slowly. The reaction was continued at a constant temperature of 35 °C with stirring for 30 min, followed by dilution with 46 mL warm de-ionized (DI) water. Subsequently, the reaction temperature was increased to 98 °C for another 30 min with stirring, and then reaction mixture was diluted by 140 mL DI water and the residual permanganate and manganese dioxide were reduced by adding 2.5 mL H_2O_2 with a change of suspension color from brown to yellow. The resulting suspension was centrifuged to collect solid product which was washed with absolute ethanol and DI water sequentially until the pH of the supernatant reached 7. Afterwards, the product was dried in a vacuum oven to obtain graphite oxide powders. Finally, homogeneous GO suspension in reddish brown was obtained through ultrasonic exfoliation of the graphite oxide dispersed in DI water for 1 h.

2.3. Preparation of GO-Ag

GO-Ag nanocomposites were prepared by reducing $AgNO_3$ with $HCOONH_4$ in presence of GO suspension [25]. Briefly, 0.4 g $AgNO_3$ was added into GO suspension pre-prepared from 0.5 g GO and mixed it uniformly. After that, 50 mL 0.5 mol L^{-1} $HCOONH_4$ aqueous solution was added drop by drop with stirring at room temperature for 1 h to induce complete reduction. After 24 h ageing, the mixture was centrifuged and washed with absolute ethanol and DI water sequentially. Then, GO-Ag nanocomposites were obtained after drying in a vacuum oven for 12 h.

2.4. Characterization

The sizes and morphologies of GO and GO-Ag were characterized by transmission electron microscopy (TEM) (JEOL-1230 microscope, Japan). The composition and bond energy information of GO and GO-Ag were investigated by X-ray photoelectron spectroscopy (XPS) (K-Alpha 1063, United Kingdom) using a Thermo Fisher Scientific Theta Probe Spectrometer equipped with Al Kα Micro gathered monochromator as the source of X-ray. Binding energy of Ag was measured by referring to the C 1s peak at 284.8 eV. Detailed spectra processing was performed by commercial Thermo Avantage software (v. 5.52, ©1999–2012 Thermo Fisher Scientific Inc.).

2.5. Bacterial culture and counting method

The bacterial strains were cultivated in Luria-Bertani (LB) fluid nutrient medium (tryptone 10 g L^{-1}, yeast extract 5 g L^{-1}, and NaCl 5 g L^{-1}) with approximately 120 rpm shaking speed at 37 °C for 24 h. Then, bacterial cells were gathered by centrifugation and washed three times to remove all residual LB. Finally, the bacterial cells were re-suspended in sterile water and diluted to achieve the desired initial concentrations containing cells 10^5–10^6 CFU mL^{-1}. All experimental mediums and glass wares were sterilized at 121 °C for 15 min with autoclave before they were used.

The antibacterial activity of GO-Ag and the microbial changes were evaluated by plate colony-counting method. Concretely, the undetermined samples were diluted by ten times gradient dilution with sterile water. After that, for the purpose of inhibiting other microorganisms and forming uniform colonies, 100 μL of each test sample was spread on a selective eosin methylene blue agar plate (for *E. coli*) or mannitol salt agar plate (for *S. aureus*), followed by cultivation at 37 °C for 24 h. Then the number of colonies on the agar plate was counted and recorded for the determination of live bacteria.

The detection limit of this method is 10 CFU mL^{-1}. Antibacterial rate was calculated using the following equation:

$$\text{Antibacterial rate}(\%) = (1 - N_t/N_0) \times 100\% \tag{1}$$

where N_0 is the initial number of bacterial colonies before each experiment and N_t is the instant colony-forming units in the sample at time t after interaction with bactericidal materials.

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