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Efficient bacteria capture and inactivation by cetyltrimethylammonium bromide modified magnetic nanoparticles

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ABSTRACT

Functionalized magnetic nanoparticles have shown great application potentials in water treatment processes especially for bacterial removal. Antibacterial agent, cetyltrimethylammonium bromide (CTAB), was employed to modify Fe_3O_4 nanoparticles to fabricate bactericidal paramagnetic nanoparticles (Fe_3O_4@CTAB). The as-prepared Fe_3O_4@CTAB could effectively capture both Gram-negative *Escherichia coli* and Gram-positive *Bacillus subtilis* from water. For both cell types, more than 99% of bacteria with initial concentration of 1.5×10^7 CFU/mL could be inactivated by Fe_3O_4@CTAB (0.5 g/L) within 60 min. Fe_3O_4@CTAB could remove more than 99% of cells over a wide pH (from 3 to 10) and solution ionic strength range (from 0 to 1000 mM). The copresence of sulfate and nitrate did not affect the bacterial capture efficiencies, whereas, phosphate and silicate slightly decreased the bacterial removal rates. However, more than 91% and 81% of cells could be captured at 10 mM of phosphate and silicate, respectively. Over 80% of cells could be removed even in the presence of 10 mg/L of humic acid. Moreover, Fe_3O_4@CTAB exhibited good reusability, and greater than 83% of cells could be captured even in the fifth regeneration cycle. Fe_3O_4@CTAB prepared in this study have great application potentials for water disinfection.

1. Introduction

Although commonly used disinfectants, such as chlorine, chloramines and ozone, could effectively eliminate pathogens in water, yet these chemicals could also cause great health risk due to the generation of more than 600 kinds of disinfection byproducts [1,2]. Moreover, antibiotic-resistant bacterial strains are gradually showing up with use of conventional antibiotic methods [3–5]. For example, Gram-negative Enterobacteriaceae with resistance to carbapenem conferred by New Delhi metallo-β-lactamase 1 (NDM-1) have been found to be multiple-drug-resistant and would become a worldwide public health problem [3]. Therefore, it is very urgent to develop efficient bacteria inactivation strategy.

Magnetic nanoparticles (MNPs), consisting of a superparamagnetic core and can be conveniently separated with magnetic field, have been widely used to remove pollutants from water [4–15]. With proper functionalization, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), which are nontoxic paramagnetic materials [16], could be employed to remove bacteria from water [4,6,7,9,12,14,17]. For instance, modified with glycopeptide antibiotics, such as vancomycin [6,7,10], and D-mannose [9], the fabricated MNPs have shown great capacity for the capture of specific bacteria. Polystyrene [18], polyethyleneimine and poly(hexamethylene biguanide) [12], and zinc-coordinated bis(dipicolylamine) [19] have also been previously employed to modify MNPs to remove bacteria. However, it should be noted that the chemicals with large molecules are commonly employed to modify MNPs in previous studies. Thus, the amounts of chemicals anchored onto the MNPs surface would be limited, affecting the bacteria capture efficiency. Moreover, complicate synthetic routes are usually needed to fabricate these functionalized MNPs. Hence, modification of MNPs with small molecules, which is capable of capture bacteria via nonselective interactions, through a simple and economical process is therefore particularly attractive.

CTAB, cationic surfactant with relative small molecules, could be easily anchored onto the surface of Fe_3O_4 nanoparticles by a facile and simple two-step transformation process. Since it is a relatively safe surfactant especially when a low dosage was employed, CTAB thus has been commonly used to modify adsorbents to remove negatively charged pollutants from water [20,21]. The positive charged CTA^+ could also interact with negative charged

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bacteria through nonselective electrostatic interaction. Therefore, modification of Fe_3O_4 particles with CTAB are expected to be capable of capture bacteria. Moreover, previous study [22] reported that through interfering with the activities of respiratory enzymes and/or the energy coupling machinery that were critical for cell growth, CTAB could cause a delay in bacterial growth at relatively low concentrations. Nakata et al. [23] found that CTAB present in suspensions could generate superoxide and hydrogen peroxide, which would inhibit the SoxS function, decrease Mn–SOD activity of *Escherichia coli* cells, and thus lead to the death of bacteria [23]. Hence, CTAB modified Fe_3O_4 nanoparticles (Fe_3O_4@CTAB) would contain capacity of both cell capture and inactivation, yet which has never been explored and thus requires investigation.

Herein, CTAB was employed to modify Fe_3O_4 nanoparticles through a simple two-step transformation process for bacteria capture and disinfection. The effects of material dosage, solution pH, ionic strength, coexisting anions, as well as natural organic matter (NOM) on the bacteria removal were systematically studied. The reusability of Fe_3O_4@CTAB was estimated with five consecutive bacteria capture and material regeneration cycles. Moreover, the mechanisms driving Fe_3O_4@CTAB to capture and inactivate bacterial cells were also discussed. The fabricated Fe_3O_4@CTAB offer a cost-effective and efficient alternative to eliminate microorganisms from water.

2. Materials and method

2.1. Materials

Suwannee River humic acid (SRHA) (Cat#2S101H, Standard II, International Humic Substances Society), which has been used as model NOM in previous studies [24–26], was employed to model NOM in this study. The molecular weight of the SRHA used in this study were 1–5 kDa. Cetyltrimethylammonium bromide (CTAB) was purchased from Sigma–Aldrich (Sigma–Aldrich, St. Louis, MO, USA). $FeCl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, $NH_3{\cdot}H_2O$, NaCl, Na_2SO_4, $NaNO_3$, Na_3PO_4, HCl, Na_2SiO_3, and NaOH used in this study were all purchased from Sinopharm Reagent Corporation Ltd. All the chemicals were analytical grade and used without further purification.

2.2. Synthesis of Fe_3O_4@CTAB

Fe_3O_4 nanoparticles were firstly synthesized through co-precipitation method [27] with slight modification. 20 mL 1 M $FeCl_2$ and 40 mL 1 M $FeCl_3$ were mixed with a stirring blade in a 150 mL flask containing 20 mL of oxygen free Milli-Q water. Ultrapure N_2 gas (99.999%) was used to create an oxygen-free environment during the synthesize process. The mixture was heated to 80 °C in a digital heating circulating water bath. Then ammonium hydroxide (28-30%) was dropped into the mixture until the pH reached around 10, and black precipitate was formed. The mixture was then stirred at 80 °C for another 20 min. After cooled to room temperature, the black precipitate was collected with a magnet and repeatedly washed with deionized water until the pH of suspension was neutral. Then CTAB was anchored onto the MNPs surface by exposing the black precipitate to 100 mL of 0.06 M CTAB solution for 30 min under sonication conditions. After that, the black precipitate was collected with magnetic decantation and washed with deionized water three times to wash away the unreacted CTAB. The nanoparticles then were dried at 80 °C under vacuum for 12 h. Fe_3O_4@CTAB nanoparticles were obtained.

2.3. Characterization of Fe_3O_4@CTAB

Powder X-ray diffraction (XRD, DMAX-2400, Rigaku, Japan) and X-ray photoelectron spectroscopy (XPS, Axis Ultra, Kratos, UK) were employed to determine the components of the fabricated nanomaterial, respectively. Fourier transformed infrared spectroscopy (FTIR) obtained from NICOLET750 (Nicolet, USA) in the 400–4000 cm^{-1} wavenumber range was recorded to confirm the modification of CTAB on the surfaces of nanoparticles. The amounts of CTAB loaded on MNPs were estimated by TOC-VCPN (Shimadzu, Japan). The size and morphology of the nanomaterial were characterized by transmission electron microscopy (TEM, Hitachi H-9000 NAR, Japan) at 300 kV. A quantum design super conducting quantum interference device (SQUID) magnetometer (MPMSXL-7 Tesla, Quantum Design, USA) was used to estimate the magnetic properties of the prepared particles. Zeta potentials of the synthesized material at varied pH conditions were measured using Zetasizer Nano ZS90 (Malvern Instruments, U.K.). It should be noted that prior to zeta potential measurement, 2 mg of Fe_3O_4@CTAB nanoparticles were dispersed in 20 mL Milli-Q water by sonication (100 W, 40 kHz) for 10 min and then adjust the solutions to different pH with NaOH or HCl. Zeta potential measurements were performed at room temperature (25 °C) and repeated 18–24 times.

2.4. Bacteria preparation

Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Bacillus subtilis* (*B. subtilis*), which have been widely used as model bacteria of Gram-negative strain and Gram-positive strain in previous studies [28,29], were employed in this study as model cells. *E. coli* was inoculated in 100 mL of Luria Broth growth medium, which consisted of 10 g/L tryptone, 5 g/L bacto-yeast extract, and 10 g/L NaCl, whereas, *B. subtilis* was cultivated in 100 mL of growth medium that containing 15 g/L tryptone, 5 g/L bacto-yeast extract, and 5 g/L NaCl. Both strains were cultivated in an incubator (shaken at 200 rpm) until the early stationary phase was reached (37 °C and 16 h for *E. coli*, 30 °C and 32 h for *B. subtilis*). Cells were then separated from the growth medium by centrifugation (4000 × *g* for 10 min at 4 °C). The obtained bacterial pellets were washed three times with sterilized physiological saline (0.9% of NaCl at pH 7.0) to remove the residual growth medium and were then re-suspended in sterilized physiological saline to obtain bacterial stock solutions with cell density of approximately 1.5×10^8 colony forming unit (CFU) per mL.

2.5. Bacteria removal experiment

2.5 mg of Fe_3O_4@CTAB was dispersed in sterilized deionized water under ultrasonication for 5 min, and then 0.5 mL of the bacterial stock solution was added into the suspension. The solution volume was ultimately fixed at 5 mL. The initial cell concentration was set to be 1.5×10^7 CFU/mL and the Fe_3O_4@CTAB dosage was 0.5 g/L. The solutions were shaken under laminar flow conditions (200 rpm) with a Reynolds number of approximately 800 [30]. At the sampling intervals, Fe_3O_4@CTAB particles were separated from the suspension with a magnet placing near to the wall of centrifuge tube, then 0.5 mL solution was sampled and analyzed for bacterial concentration via plate counting method. The bacterial capture kinetics experiments were carried out with 20 mL of bacterial suspensions (in 50-mL polystyrene centrifuge tubes) by sampling at a series of time intervals. Effect of pH was examined by adjusting the suspension with HCl or NaOH. The influence of coexisting NOM and competitive anions (sulfate, nitrate, silicate, and phosphate) on the bacteria removal were investigated by introducing the corresponding solutes into the physiological saline. To investigate the bacterial removal mechanism of Fe_3O_4@CTAB (whether Fe_3O_4@CTAB particles can only capture the cells or could also inactivate the cells), the intensively blended nanoparticle-bacteria suspension was inoculated into growth medium to yield the amounts of survived cells. The reusability of Fe_3O_4@CTAB was