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Efficient bacterial capture with amino acid modified magnetic nanoparticles

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ABSTRACT

Traditional chemical disinfectants are becoming increasingly defective due to the generation of carcinogenic disinfection byproducts and the emergence of antibiotic-resistant bacterial strains. Functionalized magnetic nanoparticles yet have shown great application potentials in water treatment processes especially for bacterial removal. In this study, three types of amino acids (arginine, lysine, and poly-L-lysine) functionalized Fe_3O_4 nanoparticles (Fe_3O_4@Arg, Fe_3O_4@Lys, and Fe_3O_4@PLL) were prepared through a facile and inexpensive two-step process. The amino acid modified Fe_3O_4 nanoparticles (Fe_3O_4@AA) showed rapid and efficient capture and removal properties for both Gram-positive *Bacillus subtilis* (*B. subtilis*) and Gram-negative *Escherichia coli* 15597 (*E. coli*). For both strains, more than 97% of bacteria (initial concentration of 1.5×10^7 CFU mL^{-1}) could be captured by all three types of magnetic nanoparticles within 20 min. With *E. coli* as a model strain, Fe_3O_4@AA could remove more than 94% of cells from solutions over a broad pH range (from 4 to 10). Solution ionic strength did not affect cell capture efficiency. The co-presence of sulfate and nitrate in solutions did not affect the capture efficiency, whereas, the presence of phosphate and silicate slightly decreased the removal rate. However, around 90% and 80% of cells could be captured by Fe_3O_4@AA even at 10 mM of silicate and phosphate, respectively. Bacterial capture efficiencies were over 90% and 82% even in the present of 10 mg L^{-1} of humic acid and alginate, respectively. Moreover, Fe_3O_4@AA nanoparticles exhibited good reusability, and greater than 90% of *E. coli* cells could be captured even in the fifth regeneration cycle. The results showed Fe_3O_4@AA fabricated in this study have great application potential for bacteria removal from water.

1. Introduction

Microbial contamination of water has been a major threat to human health. Chlorine, chloramines, and ozone are commonly used to eliminate pathogens in water. However, these chemicals could react with various constituents in natural waters to generate more than 600 kinds of disinfection byproducts (DBPs), many of which are carcinogenic (Krasner et al., 2006; Li et al., 2008). Moreover, the conventional

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antibiotic methods are becoming less efficient as the emergence of antibiotic-resistant strains (Mahmoudi and Serpooshan, 2012; Wu et al., 2013). Development of new bacteria decontamination strategy is therefore of great urgency and importance.

Capture and separation of bacteria from water with bacteria-adhesive materials has provided an alternative to eliminate the pathogens from water (Kawabata et al., 1983; Busscher et al., 2008; Akasaka and Watari, 2009; Rotem et al., 2010). Akasaka and Watari (2009) found that carbon nanotubes could effectively capture *Streptococcus mutans* and form precipitation to remove the bacteria from water. Recently, Rotem et al. (2010) reported a resin-linked oligoacyllysine bead, which could efficiently capture 3000 bacterial cells with one single bead (with size of 50–100 μm). However, the application of most of these materials would be inhibited by the difficulty in material recovery due to their relatively small sizes. Whereas, magnetic nanoparticles (MNPs), which can be conveniently separated from water by the employment of magnetic process, have been used for target bacteria detection and decontamination after functionalized with organic molecules (Honda et al., 1998; Gu et al., 2003; Lin et al., 2005; El-Boubbou et al., 2007; Kell et al., 2008; Liu et al., 2008a; Bromberg et al., 2009, 2011; Huang et al., 2010; Wen et al., 2013). Specifically, by properly modifying MNPs with biomolecules capable of selectively interacting with surface groups of bacteria, the functionalized MNPs could efficiently capture and concentrate the bacterial cells (Gu et al., 2003; Lin et al., 2005; Wen et al., 2013). However, many of such biomolecules are large molecules which would decrease their amounts anchored onto the MNPs surface and therefore affect the bacteria capture efficiency (Grossman et al., 2004; Lin et al., 2005; Kell et al., 2008; Bromberg et al., 2009). Moreover, these biomolecules such as antibody and antibiotics behave as affinity probes only for specific pathogens through hydrogen bonding (Lin et al., 2005), which might be ineffective to other bacteria. Hence, modification of MNPs with small molecules capable of participating in bioconjugation reactions through nonselective interactions seems to be particularly attractive. Huang et al. (2010) employed 3-aminopropyl triethoxy silane to attach amine groups onto the surface of tetraethyl orthosilicate coated MNPs to get amine-functionalized magnetic nanoparticles (AF-MNPs). Although the AF-MNPs could effectively capture both Gram-positive and Gram-negative bacteria through nonselective electrostatic interaction and hydrophobic interaction, the synthesis method of the AF-MNPs is too complicated and expensive. Actually, AF-MNPs could also be obtained by attaching amino acid (AA) onto the surface of MNPs through a simple and economical two-step transformation process (Unal et al., 2010). However, to date, the bacteria capture property of AA coated Fe_3O_4 nanoparticles has never been explored and thus requires investigation.

Herein, three strong positive charged amino acids, arginine (Arg), lysine (Lys), and poly-L-lysine (PLL) were firstly used to modify MNPs through a simple two-step transformation process (TST) for bacteria capture and removal. The influences of material dosage, solution pH, ionic strength, competitive anions, as well as natural organic matter (NOM) on the bacteria capture were systematically studied. Moreover, recovery and reusability of the Fe_3O_4@AA nanoparticles were examined through five consecutive bacteria capture and material regeneration cycles. The roles of electrostatic interaction, hydrophobic interaction, and hydrogen bonding in the bacteria capture process were also discussed. The AA functionalized magnetic nanoparticles offer a cost-effective and simple approach to remove pathogenic microorganisms from water.

2. Materials and method

2.1. Materials

$FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, $NH_3 \cdot H_2O$, NaCl, Na_2SO_4, Na_3PO_4, Na_2SiO_3, $NaNO_3$, L-arginine, and L-lysine used in this study were purchased from Sinopharm Chemical Reagent Co., Ltd. Poly-L-lysine was provided by Sigma–Aldrich company. All the chemicals were of analytical grade and used without further purification. Suwannee River humic acid (SRHA) (Cat#2S101H, Standard II, International Humic Substances Society), which has been previously used as model NOM (Franchi and O'Melia, 2003), was employed to model humic substances. Alginate (A2158, Sigma–Aldrich, St. Louis, MO), has also been widely employed to represent polysaccharides (Manka and Rebhun, 1982) was used to model polysaccharides in this study.

2.2. Preparation of AA modified magnetic nanoparticles

Magnetite nanoparticles were prepared and modified with Arg, Lys, and PLL to get Fe_3O_4@Arg, Fe_3O_4@Lys, and Fe_3O_4@PLL, respectively, by TST process with some modification (Tie et al., 2006; Jin et al., 2012). Briefly, 30 mL of 0.1 M $FeCl_2$ and 60 mL of 0.1 M $FeCl_3$ solutions were mixed with a mechanical stirrer in a 100-mL flask containing 20 mL of deoxygenated Milli-Q water. Ultrapure nitrogen gas was bubbled throughout the synthesis process to expel oxygen. The solution was heated to 80 °C with a digital heating circulating water bath. Ammonium hydroxide (28–30%) was then added to the solution drop by drop until the pH reached around 10, under which conditions black precipitate was formed. Then the mixture was stirred at 80 °C for 20 min and cooled to room temperature. The black precipitate was collected by magnetic decantation and washed with deionized water repeatedly until the washings were neutral. The obtained black precipitate was then equally divided into three parts for modification with three types of amino acids. The obtained black precipitates were then dispersed into 100 mL of the corresponding AA solution under ultrasonication for 30 min. It should be noted that the weight ratios of Arg and Lys to Fe_3O_4 nanoparticles were controlled at 1:1, while PLL was controlled at 0.1:1. After the sonication treatment, the obtained black precipitates were collected with magnetic decantation and washed with deionized water three times. Then the AA modified Fe_3O_4 nanoparticles could be obtained after a vacuum dry process at 80 °C for 12 h and stored in a stoppered bottle under vacuum for further use.