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APPLICATION OF MAGNETIC NANOPARTICLES IN DRINKING WATER PURIFICATION

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Abstract

Pathogenic bacteria are fetal to human health, requiring cost-effective and safe approaches to be removed from drinking water source. With poly-allylamine-hydrochloride (PAAH) stabilization, magnetic nanoparticles (MNPs) were introduced in this study to remove pathogenic bacteria by electrostatic interaction and magnet capture. High removal efficiency was achieved for four main pathogenic species, as *Escherichia*, *Acinetobacter*, *Pseudomonas* and *Bacillus*, and over 99.5% of the pathogens can be removed when the bacterial count was less than 10^5 CFU/mL. Related to various species, the MNPs have respective adhesion effects on bacterial cells, which are higher for *Acinetobacter* and *Pseudomonas*, due to the mechanisms of external cell structure and ion exchange capacity, but not the zeta potential of bacterial cell surface. With the practical application in real drinking water samples collected from reservoirs in Sheffield and Leeds, the results showed high bacteria removal efficiency (99.48%) and the total bacteria residual counts was as low as 78 CFU/mL, which met the drinking water standard of WHO (<100 CFU/mL). Further toxicity test indicated that no significant genotoxicity or cytoxicity existed in MNPs treated water, suggesting MNPs are biocompatible for safety issues in drinking water. As an effective, easy-operation and low cost technique, MNPs have bright future and great potential in practical drinking water treatment to remove pathogenic bacteria.

Key words: drinking water, magnetic nanoparticles (MNPs), pathogenic bacteria, water purification

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1. Introduction

Pathogenic bacteria exist at low concentration in drinking water, which are hard to detect but with serious threats to human health (Lewis and Gattie, 2002). According to World Health Organization (WHO, 2011), many infectious diseases are caused by pathogenic bacteria, viruses and parasites, regarded as one of the highest risks associated with drinking water safety (World Health Organization, 2011). It raises great opportunities for novel technical development in water treatment plant to achieve high bacteria removal efficiency and secure drinking water safety. The widely applied methods included filtration (Hijnen et al., 2010; Simonis and Basson, 2012), adsorption (Klein et al., 2013; Robertson et al., 2012) and coagulation (Katsoylannis and Zouboulis, 2006). Filtration is mostly found in large drinking water treatment plants in urban areas, like slow sand filtration (SSF) or bio-sand filtration (BSF) (Tellen et al., 2010).

For water treatment and disinfection in small scale or for house-hold, the membrane improvement is introduced as advanced filtration techniques for pathogens removal (Ahmed et al., 2013; Zhang et al., 2013c). Coagulation is another approach to remove bacteria by the act of precipitation in both drinking water (Pariseau et al., 2013) and wastewater treatment (Zhang et al., 2013b). Many chemical coagulants, such as aluminum sulphate ($Al_2(SO_4)_3$), ferric sulphate ($Fe_2(SO_4)_3$) and ferric chloride ($FeCl_3$), have good disinfection performance but the

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high cost restricts its application in rural areas (Moayedi et al., 2014). Thus, natural coagulants are attempted for pathogens reduction in developing countries (Khodapanah et al., 2013). The seeds of *Moringa oleifera* are widely used biocoagulants, binding the colloidal particles and allowing an electrostatic precipitation of contaminates from water (Ndabigengesere et al., 1995).

Pathogenic bacteria can be effectively removed by appropriate dosage of *M. oleifera* seeds and the normal dosage in drinking water treatment ranges from 2 to 200 mg/L. Comparing the alternative disinfection technologies, the operation cost is mostly concerned for engineering application in rural areas and developing countries (Mwabi et al., 2011), and the residual toxicity also restricts the utilization and dosage of adsorption and coagulation reagents (Ahmed et al., 2013). For instance, the residual aluminum is required less than 0.2 mg/L after aluminum sulphate coagulation to meet WHO quality guidelines (WHO, 2011). The significant toxic threat of *M. oleifera* is also reported and the hydrophobic fatty acidic components contribute to the dominant toxicity (Al-Anizi et al., 2014; Prabhu et al., 2011).

As an effective approach to functionalize biological molecules, magnetic nanoparticles (MNPs) have great potential in recognizing and targeting bacteria for drinking water purification (Huang et al., 2010; Li et al., 2013). Nevertheless, the approaches at early stage cannot achieve high bacteria removal efficiency due to the poor performance of polymer associated with MNPs. Many approaches therefore have been applied to stabilize MNPs and improve its coating efficiency on bacterial cell surface (Luis Corchero and Villaverde, 2009). One potential solution is to apply transition elements during MNPs synthesis process, such as Co (Ennen et al., 2012) and Fe (Ozdemir et al., 2012), to enhance the magnetism.

Another attempt is developed to immobilize MNPs with organic polymers to stabilize the cationic polyelectrolyte and improve the aggregation (Safarikova et al., 2009). Various biocompatible and efficient polyelectrolyte materials and their specific performances have been studied (Fakhrullin et al., 2010b; Huang et al., 2010; Zhang et al., 2011). The recent breakthrough of MNPs functionalization with poly-allylamine-hydrochloride (PAAH) helps further technical development in MNPs application in pathogenic bacteria removal from drinking water (Zhang et al., 2011). They can be applied not only in environmental monitoring (Zhang et al., 2012, 2013a), but also as a practical tool to manipulate and remote control single cell (Dobson, 2008; Lagus and Edd, 2013; Luis Corchero and Villaverde, 2009; Michan et al., 2012). PAAH is a positively charged, non-toxic and biocompatible polymer, and it has been reported with strong adhesion capacities to the cell surface. With a modified approach to functionalize MNPs with PAAH, the aqueous dispersion of MNPs is stable without aggregation for at least 6 months. PAAH functionalized MNPs has been proved to effectively functionalize living bacteria (Balkundi et al., 2009; Zhang et al., 2011), yeast (Fakhrullin et al., 2010a), algae (Fakhrullin et al., 2010b) and even human cells (Dzamukova et al., 2011). Furthermore, the remote control by magnetic field for MNPs functionalized bacteria is easy and highly efficient, indicating the significant engineering potential for pathogenic bacteria removal from drinking water (Chen et al., 2013).

This paper therefore addressed on the optimization and toxicity assessment of MNPs application in drinking water purification. The relationship between MNPs dosage and pathogenic concentration has been investigated, as well as the removal efficiency of respective bacterial species. Furthermore, the toxicity test by whole cell bioreporter indicated that no significant genotoxicity was observed after MNPs treatment, offering a safe and cost-effective approach to remove pathogenic bacteria from drinking water, especially feasible in developing countries.

2. Material and methods

2.1. Magnetic nanoparticles synthesis

As described previously (Zhang et al., 2011), chemical co-precipitation was utilized for magnetic nanoparticles synthesis, with the reaction of ferrous chloride and ferric chloride under alkaline condition. Briefly, 2.0 mL of 1 M $FeCl_3$ and 0.5 mL of 2 M $FeCl_2$ were gently mixed and stirred vigorously. 25 mL of 2.5 M NaOH solution was slowly added drop by drop, and the reaction solution was kept stirring until the dark iron oxide precipitate appeared. Keeping vortexing for 30 minutes, the oxide suspension was separated by permanent magnet and the supernatant was discarded.

The magnetic pellets were washed by sterile deionized water until the supernatant became neutral (pH=7.0). 1 mL of the MNPs suspension was then mixed together with 10 mL of 10 mg/mL aqueous poly-allylamine-hydrochloride (PAAH) to stabilize the cationic polyelectrolyte, kept sonicating for 10 minutes (40 kHz, LNGF175, Langford Electronics Ltd, UK). The PAAH-stabilized-MNPs was purified twice by centrifugation (10,000 rpm, 10 minutes) and resuspended in 1 mL sterile deionized water. Finally, the suspension passed through 0.20 μm disposable sterile filter (Millipore, USA) as the PAAH-stabilized-MNPs stock solution.

2.2. Cell cultivation and pretreatment

All the pure cultured pathogenic bacterial strains are listed in the following Table 1, which are all suggested pathogens by World Health Organization (WHO, 2011) in drinking water. The culture medium for all the strains was Luria-Bertani (LB), consisting of 25.0 g LB Broth (Merck, Germany) in 1 L deionized water and autoclaved.

The strains were inoculated from single colony on LB agar plate into LB liquid medium overnight before usage, at respective temperature of 30°C for *Acinetobacter baylyi* and *Pseudomonas putida*, and 37°C for *Escherichia coli* and *Bacillus subtilis*. The cell suspension was subsequently centrifuged at 3,000 rpm for 5 minutes and the bacterial pellets were resuspended in the same volume of sterilized water.

Table 1. Bacterial strains applied in this study

Strain	***Reference***
Acinetobacter baylyi ADP1 (BD413)	(Juni and Janik, 1968)
Escherichia coli DH5α	(Grant et al., 1990)
Bacillus subtilis 168	(Warth, 1978)
Pseudomonas putida KT2440	This study

2.3. Magnetic nanoparticles (MNPs) manipulation

The OD_{600} of all the resuspended pathogenic bacteria suspensions were measured by Synergy 2 multimode microplate reader (BioTek Instruments, Inc., USA), and the cell count was calculated in accordance with standard curve (Zhang et al., 2011). All the cell suspensions were diluted in series of 10^8, 10^7, 10^6, 10^5, 10^4 and 10^3 CFU/mL for removal efficiency assessment at different concentration. 1 mL of each cell suspension was mixed with 100 μL PAAH-stabilized-MNPs stock solution, incubated under room temperature for 10 minutes. The MNPs-associated-bacteria were separated from the supernatant, which was labeled as MNPs-free bacteria cells, by magnetic bar for 15 minutes, and then resuspended in 1.0 mL sterile deionized water.

The cell amounts of MNPs-free and MNPs-associated bacterial cells were evaluated by cell counting. The respective bacterial suspensions were stepwisely diluted from 10^2 to 10^6 times and spread on the LB agar plate. After overnight inoculation under respective temperature shown above for various strains, pathogenic bacteria concentration was counted by the colonies on the LB agar plate. The bacteria removal efficiency was assessed by dividing the count of MNPs-free cells by the total cell count (both MNPs-free and MNPs-associated bacteria).

2.4. Drinking water sample application

Thirteen real drinking water samples were collected from two places in the UK, 5 samples from Ladybower Reservoir (53°23′ N-1°43′ W, named as L1 to L5 respectively) in Sheffield and 8 from Yeadon Tarn Lake (53°52’ N-1°40’ W, named as Y1 to Y8 respectively) in Leeds.

The water samples were sealed in glass bottle and stored at 4 °C before further treatment. The procedures of MNPs functionalization and cell counting in real drinking water samples followed the same instructions as described above.

2.5. Toxicity test

The standard solutions for genotoxicity and cytoxicity assessment are 10 μM mitomycin C and 5 mM $ZnCl_2$ respectively. The water toxicity evaluation after MNPs treatment was conducted with the method described previously (Zhang et al., 2011). More precisely, the 1.0 mL of *Acinetobacter baylyi* ADPWH_recA bioreporter (Song et al., 2009) was inoculated at overnight 30°C. After 3000 rpm centrifugation for 10 minutes, the bioreporter pellet was resuspended in 10 mL of fresh LB culture medium. The 180 μL bioreporter suspension was subsequently mixed with 20 μL targeting water samples (before and after MNPs treatment), and transferred into each well of 96-well black clear bottom microplate (Corning Costa, USA).

With three replicates for each sample, the bioluminescent and OD_{600} data were measured every 10 minutes in Synergy 2 multimode microplate reader (BioTek Instruments, Inc., USA) for 6 hours incubation at 30°C. The relative bioluminescence was utilized to assess the toxic effects of water sample on ADPWH_recA bacterial activities and SOS response, by the ratio of bioluminescence to OD_{600}.

3. Results and discussion

3.1. Physical properties of magnetic nanoparticles functionalized bacteria

From previous research (Zhang et al., 2011), PAAH stabilized MNPs had a positive zeta potential as +38±6 mV, with a spherical shape and size distribution around 18±3 nm. Due to the negative charge on bacterial cell surface, PAAH stabilized MNPs had high coating efficiency of above 99.96% for *Acinetobacter baylyi.*

More interestingly, the MNPs functionalized bacteria could be easily harvested by permanent magnet from the suspensions (Fig. 1), and the capturing time is within 5 minutes. Besides, the electronic charge immobilization requires no specific MNPs or cell surface pretreatment, allowing various species suitable for the functionalization process. The results indicated the potential capability to apply MNPs in different drinking water purification to remove pathogenic bacteria with high effectiveness.

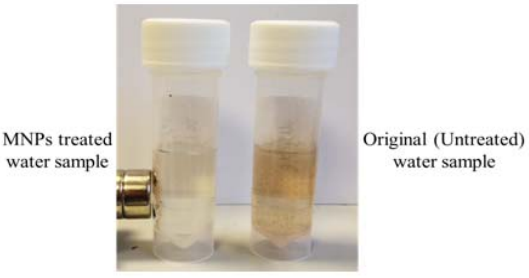

Fig. 1. MNPs treated water sample before/after magnetic capture

3.2. Bacteria removal efficiency

Four targeting pathogenic bacterial species, including *Escherichia coli*, *Acinetobacter baylyi*, *Bacillus subtilis* and *Pseudomonas putida*, were tested in this research to identify the respective MNPs removal efficiency. Considering the bacterial concentration in natural water bodies, which ranges from 10^3 to 10^6 CFU/mL, the removal test was carried out with similar cultivated bacterial concentration. The PAAH-stabilized-MNPs behaved high cohesion for all types of pathogenic bacteria, as illustrated in Fig. 2.

Significant higher removal efficiencies were observed at lower bacteria concentration, where >99.5% efficiency could be achieved when cell concentration was 1×10^4 CFU/mL or lower. At higher concentration, less removal efficiency was found for *Escherichia coli* (93.1%) and *Bacillus subtilis* (98.2%), but they remained high performance for *Acinetobacter baylyi* and *Pseudomonas putida* as 99.7% and 99.9%. Compared to the recent work where the pathogens removal efficiency was 97.4%, 95.1% and 90.1% for *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* respectively (Zhan et al., 2014), the performance in our research was much higher due to the $-NH_2$ functionalization by PAAHs (Singh et al., 2011). The existence of surface Fe_3O_4-NH_2 significantly improved the electrostatic interaction and helped the capture of bacterial cells on MNPs surface.

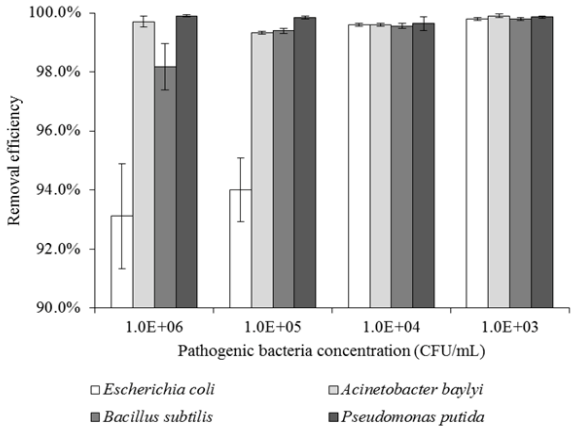

Fig. 2. Removal efficiency of different pathogenic bacteria in water samples

Some features of bacterial cell surface, such as zeta potential and chemical components, can affect the cohesion of MNPs. Previous investigations about bacterial surface hydrophobicity have identified the various zeta potential of the strains studied in this research, which was around -20 mV for *Escherichia* (Alves et al., 2010), -26 mV to -60 mV for *Acinetobacter* (Soon et al., 2011), about -15 mV for *Pseudomonas* (Roosjen et al., 2006) and -40 mV to -50 mV for *Bacillus* (Ahimou et al., 2001).

It is clear that no significant correlation is observed between bacterial zeta potential and MNPs adhesion, indicating that zeta potential is not the crucial factor affecting the MNPs removal efficiency. As investigated in previous research on the electrostatic interaction between MNPs and bacterial cells, the main reason causing the distinct coating efficiency variation was explained by two mechanisms as external cell structure (Gram-positive vs Gram-negative) and ion exchange capacities of respective cells (Terada et al., 2006). Compared to carboxyl (-COOH) and thiol (-SH) functional groups, the amine ($-NH_2$) functionalization contributed to the formation of surface complexation of metal ions (Singh et al., 2011).

The work also reported that *Escherichia* cells have less anion-exchange groups existing on the cell surface to reduce the adhesion capacities on amino polymers (Singh et al., 2011). On the other hand, Gram-positive bacteria (*Bacillus*) have thicker peptidoglycan layer and relatively lower ion exchange capacity to adhere to amino functional groups (Li and Logan, 2005), with lower removal efficiency compared to *Acinetobacter* and *Pseudomonas*.

3.3. Magnetic nanoparticles application in drinking water purification

The results of MNPs application for real drinking water purification were shown in the following Table 2. High bacteria removal efficiency (99.48%±0.12%) was achieved and there was no significant treatment difference (p-value>0.05) between the samples from Ladybower Reservoir (L1 to L5, 99.54%±0.15%) and Yeadon Tarn Lake (Y1 to Y8, 99.44%±0.18%).

Table 2. Bacteria removal efficiency in real drinking water samples

Sample	***Cell count (CFU)***		***Efficiency***
	Before	***After***	
L1	20,600	80	99.61%
L2	19,400	30	99.85%
L3	22,100	80	99.64%
L4	13,600	50	99.63%
L5	8,800	90	98.98%
Y1	11,600	120	98.97%
Y2	18,600	160	99.14%
Y3	19,600	30	99.85%
Y4	22,400	70	99.69%
Y5	8,200	120	98.54%
Y6	20,900	90	99.57%
Y7	33,800	30	99.91%
Y8	32,600	60	99.82%

Note: Samples L1 to L5 refer to those collected from Ladybower Reservoir and Y1 to Y8 from Yeadon Tarn Lake.

3.4. Toxicity estimation after MNPs treatment

Compared with the total bacterial count before MNPs treatment (19,400±2,170 CFU/mL), the residual bacteria counts were only 78±11 CFU/mL, which were at acceptable level for drinking water

purification (<100 CFU/mL, WHO). The pathogens reduction efficiency was slightly lower than that of the four pure cultured strains when the bacterial concentration was at 10^4 CFU/mL level, because MNPs could also adsorb the suspended solids and other organic matters in real drinking water and therefore lost part of electrostatic capacities.

No significant genotoxicity or cytoxicity was observed for MNPs treated water samples in further toxicity assessment, as illustrated in Fig. 3. Without any toxic impact in the negative control treatment, the bioluminescence of ADPWH_recA was 3,251±318 RLU (relative luminescent unit). Exposing to $ZnCl_2$ of 5 mM or mitomycin C of 10 μM, the bioluminescent response of ADPWH_recA showed significant inhibition and excitation, with 54% reduction and 1.49 times increase, respectively. The results fitted with the previous investigation that the response of ADPWH_recA could distinguish the cytoxicity and genotoxicity by the relative response ratio (Song et al., 2009). The bioluminescent signals of all the water samples before and after MNPs treatment ranged from 2,974 RLU to 3,525 RLU, with no significant difference from the negative control. It indicated that no cytoxicity or genotoxicity was observed after MNPs treatment and the MNPs purified water samples were drinkable without toxic risk.

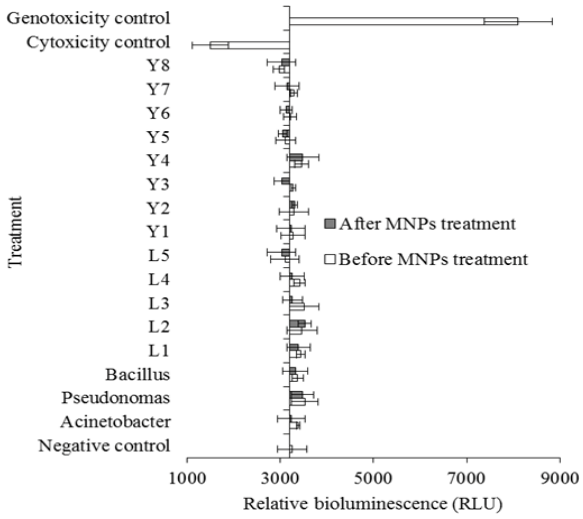

Fig. 3. Genotoxicity and cytoxicity test for water sample before and after MNPs treatment. Negative control refers to the exposure of ADPWH_recA to deionized water, and cytoxicity and genotoxicity control represent the exposure to $ZnCl_2$ (5 mM) and mitomycin C (10 μM), respectively. All the samples testing were applied by dosing water sample or supernatant after MNPs treatment to ADPWH_recA

Non-modified MNPs were reported with both cytoxicity and genotoxicity to bacterial and mammalian cells (Brunner et al., 2006). It therefore raised the concerns on the evaluation of long term toxicity impacts of MNPs on pathogens removal in drinking water and public health (Boxall et al., 2007). The surface functionalization with some silica (Kim et al., 2006) and polymers (Xia et al., 2009) can significantly reduce its toxicity. Recent work has also proved that on the PAAH-stabilized-MNPs did not affect the microbial community activities (Zhang et al., 2015), suggesting limited toxic impacts during water treatment process and in the water supply pipeline.

4. Conclusions

The application of magnetic nanoparticles application in water purification to remove bacteria from drinking water samples was discussed in this paper. The results demonstrated that PAAH stabilized MNPs were powerful tools to remove pathogenic bacteria from drinking water with high efficiency and no significant toxicity.

MNPs are therefore suitable for the removal of various pathogenic bacteria, including *Escherichia*, *Acinetobacter*, *Pseudomonas* and *Bacillus*. Compared to other disinfection technologies, MNPs disinfection is cost-effective and easy to operate, with bright future for its engineering application. The features of MNPs address the challenges of drinking water safety in rural areas of developing countries where are lack of resources and appropriate technology in water treatment. It is particularly suitable for small scale water treatment systems serving a population of between 500-1000 people and is an ideal emerging technology to provide clean water to these areas.

With the recent trends of environmental monitoring via MNPs for contamination measurement and pathogens identification, it has more potential future to be applied in drinking water sample for pathogenic bacteria removal and water quality assessment at the same time.

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