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Carboxymethyl cellulose coating decreases toxicity and oxidizing capacity of nanoscale zerovalent iron

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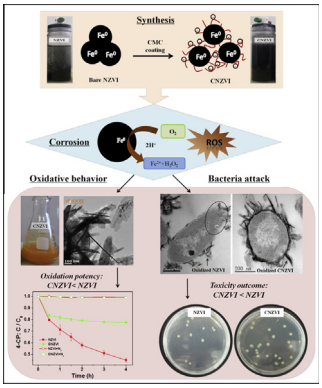
HIGHLIGHTS

- CMC-coating mitigated NZVI's toxicity to *Agrobacterium* sp. PH-08.
- CNZVI exhibited good dispersity, enhancing physical contact with PH-08.
- Oxidative potential of NZVI was inhibited by CMC introduction.
- First report on how CMC-coating affects toxicity and corrosion behavior of NZVI.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanoscale zerovalent iron (NZVI) with modified surface via coating with organic stabilizers has been documented with enhanced colloidal stability and dispersity. Therefore, the expanded application potential and accompanying intrinsic exposure of such nanoparticle can be anticipated. In our study, carboxymethyl cellulose (CMC)-stabilized NZVI (CNZVI) exerted minimized oxidative stress response and slower disruption of cell membrane integrity, resulting in mitigated cytotoxicity towards bacteria *Agrobacterium* sp. PH-08 as compared with the uncoated counterpart. The corrosive oxidation of both nanoparticles in oxygenic water provided a better understanding of coating effect. The decreased oxidative degradation of probe 4-chlorophenol with CNZVI than NZVI implicated a weaker oxidizing capacity, which might overweight massive adhesion-mediated redox damage and explain the different exposure outcome. However, enhanced evolution of iron oxide as well as the promoted production of hydrogen peroxide adversely demonstrated CMC-coating facilitated iron corrosion by oxygen, suggesting CMC was most likely to act as a radical scavenger and compete with organics or bacteria for oxidants. Moreover, XRD, XPS and TEM results showed that the spherical NZVI was oxidized to form needle-shaped iron oxide-hydroxide (γFeOOH) with no detectable oxidative stress for PH-08, alleviating worries regarding exotoxicological impact of iron nanotechnology.

1. Introduction

Nanoscale zerovalent iron (NZVI) has been widely used as an inexpensive and environmentally friendly reducing agent for the decomposition of various halogenated and nitrosubstituted pollutants. Therefore, they have received extensive attention for both in situ and ex situ environmental remediation (Choe et al., 2001; Naja et al., 2008; Crane and Scott, 2012). However, field-scale application of engineered NZVI has been limited to shallow aquifers due to easy particle agglomeration and subsequently weak delivery capacity through porous media (Phenrat et al., 2007;

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Wang et al., 2010). In order to enhance stability and mobility of nanoparticles, organic stabilizers were attached onto the surface of such materials to provide steric hindrance and/or electrostatic repulsion (He et al., 2007; Li et al., 2010). Various synthetic practices using polymers or electrolytes, such as polyacrylic acid (Cirtiu et al., 2011), Triton X-114 (Sayles et al., 1997), sodium carboxymethyl cellulose (CMC) and starch (He and Zhao, 2005, 2007), have been classified as promising candidates for rapid and effective pollutant-cleanup process.

Among a number of trials, CMC has gained great insight as a stabilizing agent since CMC-stabilized NZVI (CNZVI) exhibited relatively better dispersity (Wang et al., 2010; Cirtiu et al., 2011) and reactivity (He and Zhao, 2008; Naja et al., 2008) over many other stabilized forms. Moreover, many substantial features, like low cost, high water solubility and biocompatibility, are also conducive to its practical applicability. It is thereby predictable to witness a booming prospect of CNZVI owing to its superior physicochemical properties. Despite its underlying widespread application, limited information regarding fate and toxicological effect of such particle is within our reach.

Recent studies have demonstrated fairly strong oxidation potency of NZVI in oxygenic water to generate highly toxic reactive oxygen species (ROS) (Keenan and Sedlak, 2008; Kang and Choi, 2009), which further led to severe inactivation of living organisms, including bacteria (Lee et al., 2008), fungus (Diao and Yao, 2009) and virus (You et al., 2005). Although there is growing interest in nanotoxicity, current studies predominantly focus on behaviors of bare NZVI over its stabilized form. It is presumed that surface modification of nanoparticles might alter their physicochemical interaction with organisms and influence their toxicity and bioavailability. Li et al. (2010) and Chen et al. (2011) reported that NZVI coated with polyelectrolytes or natural organic matter (NOM) mitigated bactericidal activity of NZVI to *Escherichia coli* (*E.coli*), mainly because those supports prevented the physical contact of particles to bacteria via electrosteric repulsive force. It is therefore possible that CMC stabilization might affect specific particle characteristics of NZVI, rendering unintended ecological consequences.

The overall objective of present study was to assess the impact of CMC-coating on the bactericidal properties of NZVI towards bacteria *Agrobacterium* sp. PH-08. On this basis, chemical assay was combined with microscopic technique to characterize the bacteria cellular response as well as corrosion behavior of nanoparticles. Furthermore, oxidative degradation of probe 4-chlorophenol (4-CP) was monitored along with anaerobic reductivity towards hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to investigate how CMC-coating was linked to reactivity and toxicity expression. To our knowledge, little quantitative information has been recorded systematically on this topic aside from our study.

2. Materials and methods

2.1. Chemicals

$FeSO_4{\cdot}7H_2O$, $NaBH_4$, CMC250k, 4-CP, RDX, 1,10-phenanthroline, N,N-dethyl-1,4-phenylene-diamine (DPD), peroxidase from horseradish (type VI-A, 1500 units/mg solid), dibenzofuran (DF), and 2′,7′-dichlorofluorescein diacetate (DCFH-DA) were purchased from Sigma–Aldrich. Minimal salt medium (MSM) was prepared as described by Kim et al. (2007).

2.2. Medium and microorganism

The gram-negative strain *Agrobacterium* sp. PH-08 (GenBank JN862809) that we maintain in our laboratory was used in this study. PH-08 culture was routinely grown in MSM with 5 mM DF as a carbon source in a dark incubator (160 rpm, 30 °C). The bacteria was harvested by centrifugation, washed thrice with sterile distilled water, and resuspended in sterile water to make PH-08 stock.

2.3. Synthesis and characterization of nanoparticles

NZVI was synthesized following borohydride reduction approach as reported by our previous work (Bokare et al., 2010). CNZVI was prepared using $NaBH_4$ to reduce 200 mL Fe-CMC mixture ([Fe] = 2.5 g/L, [CMC] = 0.5 g/L) at a BH_4^-/Fe^{2+} molar ratio of 2.0 (He and Zhao, 2007). Oxidized particles were collected after exposure in oxygenic water for desired time.

The size and morphology of nanoparticles were imaged on a transmission electron microscope (TEM, JEM 2200FS with Image Cs-corrector, Japan) operated at 200 kV. X-ray diffraction (XRD) analysis was obtained using Cu Kα radiation on a MXP18 HF diffractometer (MAC Science Co., Japan). Fourier transfer infrared spectrometer (FT-IR, Nicolet 740 spectrometer) was employed to identify the polymer on particle surface. Chemical composition of nanoparticles was investigated with a VG Scientifics ELSCALAB 250 X-ray photoelectron spectroscopy (XPS) with Al Kα radiation.

2.4. Toxicity assessment

For bacterial toxicity studies, strain PH-08 was incubated with particle suspensions (160 rpm, 30 °C). At selected time intervals, bacterial culture was spread onto nutrient agar (NA) medium plates and incubated at 30 °C for 24 h. Cell viability assay in terms of viable bacterial colony forming units (CFU mL^{-1}) was counted. A confocal fluorescent microscope (Olympus FV1000) was used to assess the in vivo oxidative response by DCFH-DA fluorescence method as described by Xu et al. (2009).

To prepare TEM specimens of PH-08, control and treated cell pellets were fixed in 2% formaldehyde overnight, dehydrated sequentially in 30%, 50%, 70%, 80%, 90% and 100% ethanol. The cells were then embedded into EMBed 812 and propylene oxide, followed by being sectioned on an ultra-tome for TEM (Hitachi-7600) analysis.

2.5. Degradation of 4-CP and RDX

Batch experiments for 4-CP oxidation were conducted in 100 mL glass reactors under aerobic condition (exposed to air). To initiate reactions, nanoparticles were added into 4-CP solution with adjusted pH ranging from 2.5 to 7. At selected time intervals, 1 mL aliquot was withdrawn and added into 1 mL methanol to quench Fenton reaction, followed by filtrating through a 0.45 μm filter prior to analysis.

To evaluate CMC-coating effect on reductive reactivity under anaerobic condition, nanoparticles were mixed with deoxygenated RDX solution in 30 mL glass vials before sealing with Teflon-coated cap. One bottle was sacrificed for each time measurement.

4-CP and RDX were quantified by HPLC (Agilent 1100) equipped with a Luna C-18(2) column (150 × 4.6 mm, 5 μm, Phenomenex) and a diode-array detector. Eluent composition was 0.1% phosphoric acid and acetonitrile (6:4 v/v) for 4-CP, methanol and water (5:5 v/v) for RDX. Fe^{2+} and H_2O_2 were analyzed by the 1,10-phenanthroline method (Fortune and Mellon, 1938) and colorimetric DPD method (Bader et al., 1988), respectively.