ABSORBED POLYMER CHANGES CORROSIVE REACTIVITY OF NANOSCALE ZEROVALENT IRON AS WELL AS ITS TOXICITY TO BACTERIA *Agrobacterium* sp. PH-08

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Introduction

Nanoscale zerovalent iron (nFe^0) has been well documented with great reducing potency for the decomposition of a wide range pollutants containing chlorinated and nitrosubstituted entities. Thereby it has received much attention for both in situ or ex situ remediation of contaminated groundwater and soil [1, 2]. However, due to easy particle agglomeration and subsequent weak transportation and delivery capacity through porous media, field scale application of such nanoparticle has been limited to shallow aquifers [3, 4]. In order to prevent aggregation and enhance colloidal stability and mobility of nFe^0, organic stabilizers could be attached onto the surface of such nFe^0 to provide electrostatic repulsion, and steric or electrosteric stabilization [5, 6] . Among various trials, carboxymethyl cellulose (CMC) has gained great insight as a stabilizing agent since CMC-stabilized nFe^0 (CMC-nFe^0) exhibited fairly good dispersity as well as reactivity over many other stabilized forms [5, 7].

The increased use of nFe^0-related nanoparticles freely released to aquatic environments has raised elevated concerns about human health and environmental safety. Recent studies have demonstrated the strong oxidation potency of nFe^0 in oxygenated water to generate reactive oxygen species (ROS) through Fenton's chemistry, which further led to severe inaction of living organisms, including bacteria [8], fungus [9] and also virus [10]. Although there is growing interest in nanotoxicity, recent studied predominantly focus on the behaviors of bare iron species other than the stabilized form. It is presumed that surface modification of nanoparticles might alter their physical interactions with microorganisms and change the bioavailability [11]. The objective of our study was to assess the impact of CMC-coating on the bactericidal properties of nFe^0 towards bacteria *Agrobacterium* sp. PH-08.

Materials and methods

nFe^0 and CMC-stabilized nFe^0 were freshly prepared by the borohydride reduction approach in the presence or absence of CMC following the methods reported by our previous work [12] and He and Zhao [13]. Fourier transfer infrared spectrometer (FT-IR, Nicolet 740 spectrometer) was employed to discern the polymer on the surface of nanoparticles. The gram-negative bacterial strain, namely *Agrobacterium* sp. PH-08 (Genebank Accession No. JN862809) that we isolated and maintained in our laboratory was used to test the toxicity of the two types of nanoparticles. The feasibility of the surface modified particles to generate oxidants was evaluated by means of probing technique; 4-chlorophenol (4-CP) was selected as the target probe compound since it has been documented to undergo hydroxyl radical-mediated pathway in nFe^0/air/water system in the literature [14].

Results and Discussion

1) FTIR analysis

FT-IR spectroscopy was used to obtain information on the interaction between CMC and particles. The resemblance in the spectral features of free CMC and CMC-stabilized nFe^0 confirmed successful coating of CMC onto the surface of nanoparticles (Fig. 1). Spectra for CMC-stabilized nFe^0 revealed bands at 1437 and 1638 cm^{-1} (Δ = 201 cm^{-1}), corresponding to characteristic symmetric and asymmetric stretching of carboxylate (COO^-), respectively, reflecting that monodentate chelating was the primary mechanism over other complexation, for binding COO^- group to iron.

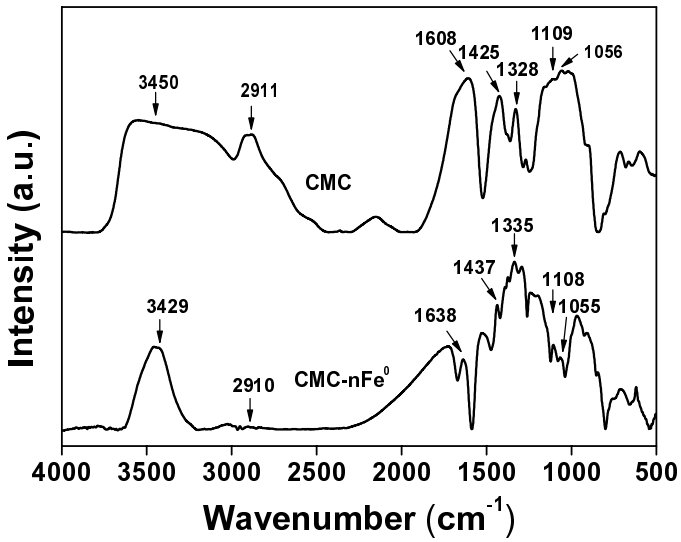

Fig. 1. FTIR spectra of CMC and CMC-stabilized nFe^0

2) Effects of nanoparticles to cell viability of PH-08

Nanotoxicity was estimated in the inhibition of colony forming units (CFU) of *Agrobacterium* sp. PH-08 with different amounts of particles (0.25 to 0.5 g/L) (Fig.2). For CMC-nFe^0, high toxic potential was observed in decreased CFU number (27.6 vs 0.8% survival after 2 h). Moreover, CMC modification reduced the toxicity of nanoparticle to PH-08; when exposed to same amount (0.25 g/L), PH-08 strain was more tolerant to CMC-nFe^0 than the uncoated form (65.1 vs 7.8% survival after 1 h).

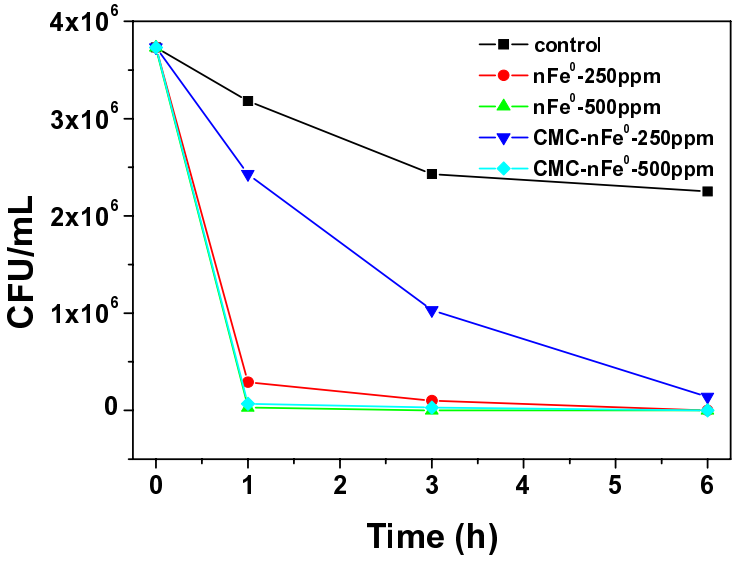

Fig. 2. Effect of bare and CMC-stabilized nFe^0 on cell viability of *Agrobacterium* sp. PH-08.

3) Mechanistic implications about CMC-mitigated toxicity

In situ generation of reactive oxygen species (ROS) through surface iron corrosion by oxygen was one great contributor to nanotoxicity of nFe^0. 4-Chlorophenol (4-CP) was chosen as the probe to evaluate feasibility of both particles to generate oxidants in aerobic condition since it could not be reduced in Fe^0/N_2 system. As shown in Fig. 3, the efficiency was detected highest at pH 3 and decreased at higher/lower pH levels. At pH 3, 63.0%

4-CP was degraded by nFe^0 (1 g/L) while the efficiency decreased to 52.1, 34.9, 29.6, 27.8 and 29.3% under pH 2.5, 4, 5, 6 and 7, respectively. However, after CMC introduction, decreased degradation was obtained under all pH ranges from 2.5 to 7 (44.9, 46.8, 32.9, 27.0, 25.2, 26.0%), implicating a weaker capacity of CMC-nFe^0 to generate oxidants, which might contribute to different toxicity outcome.

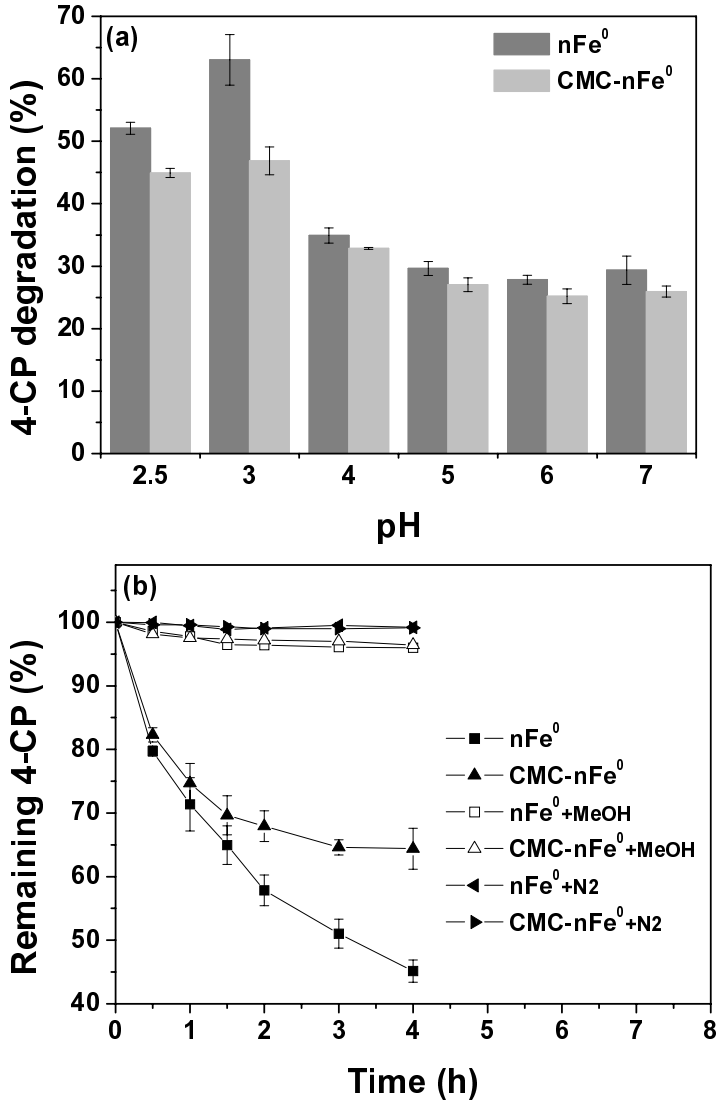

Fig. 3. (a) pH and (b) time dependence of 4-CP degradation by iron nanoparticles

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