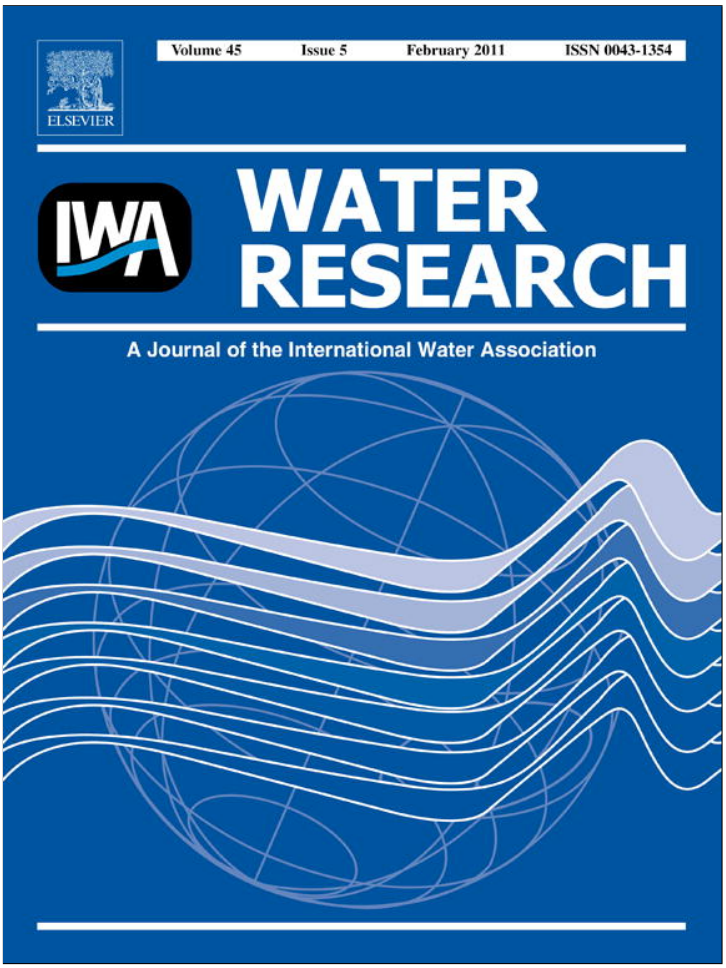

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Effect of natural organic matter on toxicity and reactivity of nano-scale zero-valent iron

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ABSTRACT

Nano-scale zero-valent iron (NZVI) particles are increasingly used to remediate aquifers contaminated with hazardous oxidized pollutants such as trichloroethylene (TCE). However, the high reduction potential of NZVI can result in toxicity to indigenous bacteria and hinder their participation in the cleanup process. Here, we report on the mitigation of the bactericidal activity of NZVI towards gram-negative *Escherichia coli* and gram-positive *Bacillus subtilis* in the presence of Suwannee River humic acids (SRHA), which were used as a model for natural organic matter (NOM). *B. subtilis* was more tolerant to NZVI (1 g/L) than *E. coli* in aerobic bicarbonate-buffered medium. SRHA (10 mg/L) significantly mitigated toxicity, and survival rates after 4 h exposure increased to similar levels observed for controls not exposed to NZVI. TEM images showed that the surface of NZVI and *E. coli* was surrounded by a visible floccus. This decreased the zeta potential of NZVI from −30 to −45 mV and apparently exerted electrosteric hindrance to minimize direct contact with bacteria, which mitigated toxicity. H_2 production during anaerobic NZVI corrosion was not significantly hindered by SRHA ($p > 0.05$), However, NZVI reactivity towards TCE (20 mg/L), assessed by the first-order dechlorination rate coefficient, decreased by 23%. Overall, these results suggest that the presence of NOM offers a tradeoff for NZVI-based remediation, with higher potential for concurrent or sequential bioremediation at the expense of partially inhibited abiotic reactivity with the target contaminant (TCE).

1. Introduction

Nano-scale zero-valent iron (NZVI) is a valuable material for *in-situ* remediation of aquifers contaminated with oxidized priority pollutants (Klimkova et al., 2008; Zhang, 2003), such as chlorinated solvents (Liu et al., 2005; Song and Carraway, 2005), hexavalent chromium (Xu and Zhao, 2007), and nitrate (Choe et al., 2000; Yang and Lee, 2005). However, as a strong reductant (E_h = −440 mV) with a relatively high specific surface area, NZVI might be toxic to indigenous bacteria and hinder their participation in the cleanup process (Diao and Yao, 2009; Xiu et al., 2010b). For example, NZVI had a bactericidal effect on *Escherichia coli* (Lee et al., 2008; Li et al., 2010), which was not observed with other types of iron including maghemite nanoparticles, micro-scale ZVI, and Fe^{3+} ions (Auffan et al., 2008; Lee et al., 2008).

When NZVI is injected into the subsurface for groundwater remediation (usually as a slurry at concentrations from 1.9 g/L

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(Zhang, 2003) to 10 g/L (Henn and Waddill, 2006)), it may interact with dissolved natural organic matter (NOM), which is one of the most abundant materials on earth and exists in natural waters in the range of a few mg/L to a few hundred mg/L C (Wall and Choppin, 2003). NOM usually includes a skeleton of alkyl and aromatic units with functional groups such as carboxylic acids, phenolics, hydroxyls, and quinones. Accordingly, NOM is known to readily adsorb to colloidal particles and bind with NZVI to enhance its mobility in water or porous media (Johnson et al., 2009). NZVI coated with humic acid showed minor toxicity to *E. coli* (Li et al., 2010). However, the effect of NZVI in the presence or absence of NOM on gram-positive bacteria (such as *Bacillus subtilis*, which is ubiquitous in the environment) has not yet been addressed in the literature. Furthermore, NOM is known to shuttle electrons for iron reducing bacteria, and could also transfer electrons in abiotic reactions. Therefore, it is important to understand how NOM affects NZVI's performance and its toxicity to bacteria that might participate in the cleanup process.

Although it is known that some nanoparticles can affect biological systems at cellular, sub-cellular and protein levels (Farre et al., 2009; Klaine et al., 2008), the mechanism of NZVI toxicity towards bacteria remains unclear. A variety of toxicity mechanisms have been proposed (Nel et al., 2009), including disruption of the cell membrane integrity (Fang et al., 2007), interference with respiration (Lyon et al., 2008), and damage of DNA or enzymatic proteins caused by released metal ions from NPs (Gogoi et al., 2006). Li et al. (2010) found that humic acid associated with NZVI decreased NZVI's toxicity to *E. coli*. They proposed that electrosteric repulsions afforded by the adsorbed NOM decreased NZVI adhesion to *E. coli* which decreased its toxicity. Aside from this study with only one type of organism, little quantitative information has been published on how NOM affects the reactivity, corrosion and toxicity of NZVI.

This paper addresses the effect of Suwannee River humic acids (SRHA), a commonly used model for NOM, on the bactericidal properties of NZVI to bacteria with different cell wall composition and morphology (i.e., the gram-negative bacterium, *E. coli* and the gram-positive bacterium, *B. subtilis*). Solution pH was controlled and zeta potential was measured along with TEM imaging to investigate how NOM affects NZVI toxicity. Abiotic reactivity was also assessed by quantifying TCE dechlorination kinetics and cathodic H_2 production (with known biostimulatory potential (Oh et al., 2001; Till et al., 1998; Weathers et al., 1997)) to assess potential tradeoffs associated with NOM–NZVI interactions.

2. Materials and methods

2.1. Chemicals

NZVI particles were obtained from Toda Kogyo Corporation, Onoda, Japan. According to the TEM images, the NZVI consisted of irregularly shaped particles ranging in size from 5 to 100 nm with a median radius of about 50 nm. The Fe^0 content in these NZVI particles was about 40%. A stock solution of 10 g/L NZVI containing 2 mM sodium bicarbonate ($NaHCO_3$) was prepared in an anoxic chamber. Suwannee River humic acid (SRHA II, International Humic Substances Society, Atlanta, Georgia) was used as model NOM without further pretreatment. Stock solutions were prepared by dissolving 1 g/L SRHA in sterile deionized water. A stock solution was freshly prepared for 1 month use and kept at 4 °C in the dark.

TCE (99%) was purchased from Sigma–Aldrich (St. Louis, MO) and $NaHCO_3$ was obtained from Fisher Scientific (Fair Lawn, NJ). Ultra-high purity gases (helium, nitrogen, hydrogen, *etc.*) were purchased from Matheson Tri-gas (Houston, TX). All chemicals used were reagent grade or better unless otherwise specified.

2.2. Bacteria

The gram-negative *E. coli* (ATTC strain 10798) and the gram-positive *B. subtilis* CB310 (courtesy of Dr. Charles Stewart, Rice University, Houston, TX) were used as model bacteria in inhibition experiments (Adams et al., 2006). *E. coli* (or *B. subtilis*) was inoculated in 30 mL of LB Broth medium (Difco Co.) and grown at 37 °C overnight. The bacteria were harvested by centrifugation at 4000*g* for 5 min, washed three times with 2 mM bicarbonate solution (pH 8.10), and re-suspended in 30 mL bicarbonate buffer to make the bacteria stock solution.

2.3. Exposure of E. coli and B. subtilis to NZVI

NZVI (10 g/L) was ultrasonicated for 1 min and added to 20 mL bacteria suspension (2–3 × 10^6 colony forming units (CFU)/mL) to make a final NZVI solution of 1 g/L under aerobic conditions. SRHA was added to achieve a final concentration of 10 mg/L. Sodium bicarbonate was chosen as a natural buffer. All reactors were shaken on a Labnet Orbit Shaker (Labnet, USA) at 200 rpm at 22 °C. Bacterial controls were prepared similarly but without NZVI or SRHA. Exposure experiments were conducted in triplicate: (i) *E. coli* or *B. subtilis* with 1 g/L NZVI; (ii) *E. coli* or *B. subtilis* with 10 mg/L SRHA; and (iii) *E. coli* or *B. subtilis* with 1 g/L NZVI plus 10 mg/L SRHA.

2.4. Toxicity assessment

Viable bacterial concentrations were determined after 0, 1, 2, and 4 h exposure by the spread plate method (Adams et al., 2006). Briefly, triplicate samples were plated on LB agar plates, incubated at 37 °C for 12 h, and the colony forming units (CFU) were counted. The bacteria survival was expressed as N/N_0 (%), where N and N_0 are the remaining and initial numbers of live bacteria (CFU/mL), respectively.

2.5. TCE degradation and cathodic H_2 generation

The effect of SRHA on NZVI reactivity towards TCE and cathodic H_2 generation in the absence of TCE were assessed separately in 250-mL serum bottles containing 100 mL bicarbonate buffer (2 mM). Two sets of experiments were conducted simultaneously in triplicate for TCE degradation: (i) TCE (20 mg/L, 15.2 μmol) with 1 g/L NZVI alone; and (ii) TCE with 1 g/L NZVI and 10 mg/L SRHA. Buffer solutions were sparged with ultra-high purity nitrogen gas for 20 min prior to NZVI and SRHA addition, and shaken at 200 rpm at 22 °C

throughout the experiment. A negative control without NZVI and SRHA was run to discern volatilization and adsorption losses. The concentration of TCE and its byproducts were measured by GC-FID over time as described below. Cathodic H_2 generation tests were set up similarly without the addition of TCE.

2.6. Analytical methods

The pH and oxidation–reduction potential (ORP) of reacting solutions were measured with a Basic pH Meter (Denver Instruments, CO, USA). Zeta potentials of bacteria and NZVI were measured using a Zeta PALS particle analyzer (Brookhaven Instruments, Holtsville, NY). Measurements were performed in 2 mM bicarbonate solution at pH 8.10 in the presence and absence of 10 mg/L SRHA. The temperature was maintained at 22 °C for all measurements.

Headspace samples (100 μL) were withdrawn from each reactor to analyze for TCE, using a GC (HP5890, MN, USA) equipped with a flame ionized detector (FID). Separation was achieved with a packed column (6 ft. × 1/8 in o. d. 60/80 carbopack B/1% SP-1000, Supelco). Analysis of H_2 was conducted by direct injection of headspace samples (200 μL) into a GC (HP 6890) equipped with a thermal conductivity detector (TCD) and a packed column (H9-Q 60/80 9 ft. 2.0 mm ID 1/8 in OD, ResTek, PA, USA). The detailed analytical procedures for TCE and H_2 analysis were described previously (Xiu et al., 2010b).

2.7. Transmission electron microscopy (TEM)

To examine the interaction of SRHA with *E. coli* and NZVI in bicarbonate buffer, NZVI particles and *E. coli* were added separately to 10 mg/L SRHA and shaken at 200 rpm at 22 °C for 1 h. A 5 μL sample of the suspension was deposited on a 400-mesh copper grid (Ultrathin carbon type-A, Ted Pella Inc., Redding, CA) and air-dried. Imaging was performed by TEM using a JEOL 1230 (JEOL, Tokyo, Japan) operated at 120 kV (SEA, Shared Equipment Authority, Rice University, Houston, TX).

2.8. Statistical analyses

Whether differences between treatments were statistically significant was determined using Student's *t*-test at the 95% confidence level. All measurements are reported as mean ± one standard deviation.

3. Results and discussion

3.1. Bacteria exposure to NZVI

Although NZVI was ultrasonicated to disperse the particles, significant aggregation occurred (especially in the absence of SHRA). TEM images showed that the size of the aggregates ranged from about 50 nm to 5 μm in the presence of SRHA (Supporting Information [SI], Fig. 1A), and were larger than 5 μm without SRHA ([SI], Fig. 1B).

B. subtilis was more tolerant to NZVI than *E. coli* (80.2 ± 6.5% vs. 35.9 ± 5.5% *survival* after 1 h incubation). The higher negative charge of *B. subtilis* compared to *E. coli* (−32.5 versus −26.9 mV) probably contributed to higher electrostatic repulsion and reduced toxicity. Whether its thicker gram-positive cell wall (composed of a relatively thick (20–80 nm) peptidoglycan layer (Madigan et al., 2006) confers additional protection remains to be determined.

SRHA (10 mg/L) significantly mitigated NZVI toxicity, increasing bacterial survival to similar levels as unexposed controls after 1 h incubation (96.3 ± 3.9% vs. 97.9 ± 1.8% for *E. coli* (Fig. 3A), and 85.1 ± 4.4% vs. 89.1 ± 6.4% for *B. subtilis* (Fig. 3B)). The positive effect of SRHA was more pronounced after 4 h exposure, increasing *E. coli* survival from 3.7 ± 0.5% to 84.2 ± 3.0% and *B. subtilis* survival from 12.4 ± 2.6% to 69.8 ± 3.0%. The type and concentration of humic acids and other types of NOM can vary widely in natural systems, and accordingly mitigate NZVI toxicity to different extents than observed in these experiments.

3.2. Effect on pH, ORP and zeta potential

NZVI (Fe^0) anaerobic corrosion in water can lead to an increase in pH:

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^- \quad (1)$$

However, the pH of the solution (1 g/L NZVI with/without SRHA) remained stable (at pH 8.10 ± 0.10) in the 2 mM bicarbonate buffer, which is within the favorable pH domain for

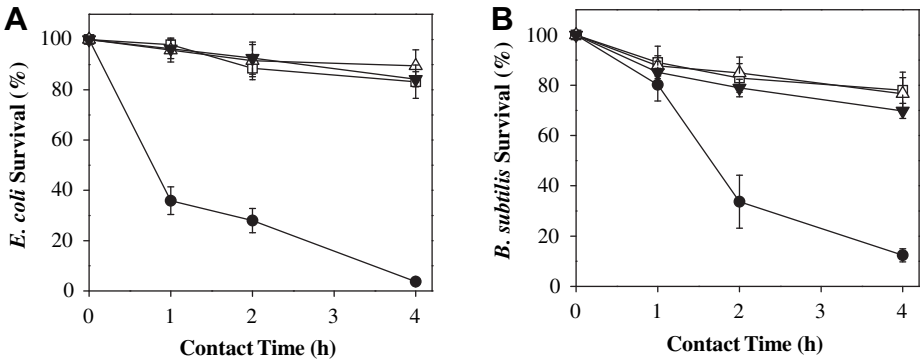

Fig. 1 – Mitigation of NZVI toxicity to (A) *E. coli* and (B) *B. subtilis* by SRHA in 2 mM bicarbonate medium, pH = 8.10 (Symbols: □, control; ●, 1 g/L NZVI; △, 10 mg/L SRHA; ▼, 1 g/L NZVI + 10 mg/L SRHA).

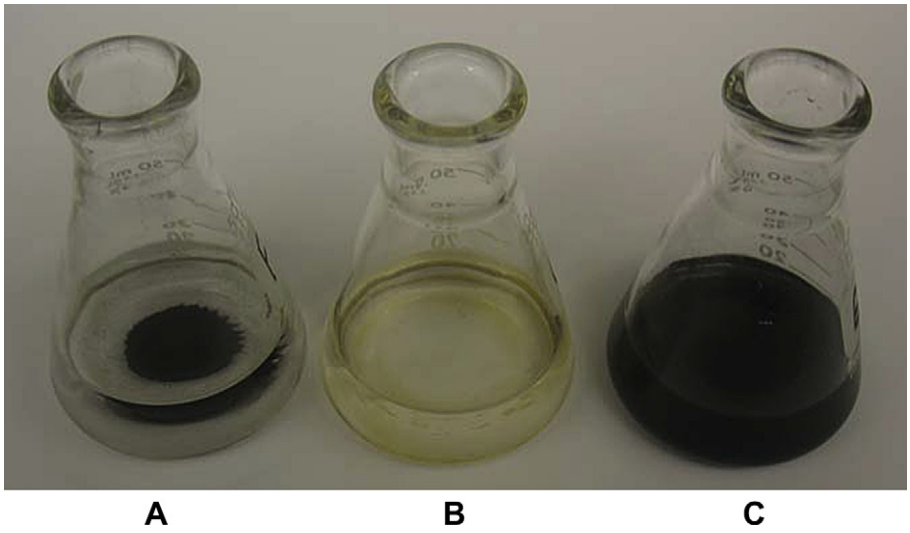

Fig. 2 – Enhanced NZVI dispersion by SRHA after shaking for 1 h at 200 rpm (A) NZVI (1 g/L) alone; (B) SRHA (10 mg/L) alone; (C) NZVI (1 g/L) in the presence of SRHA (10 mg/L).

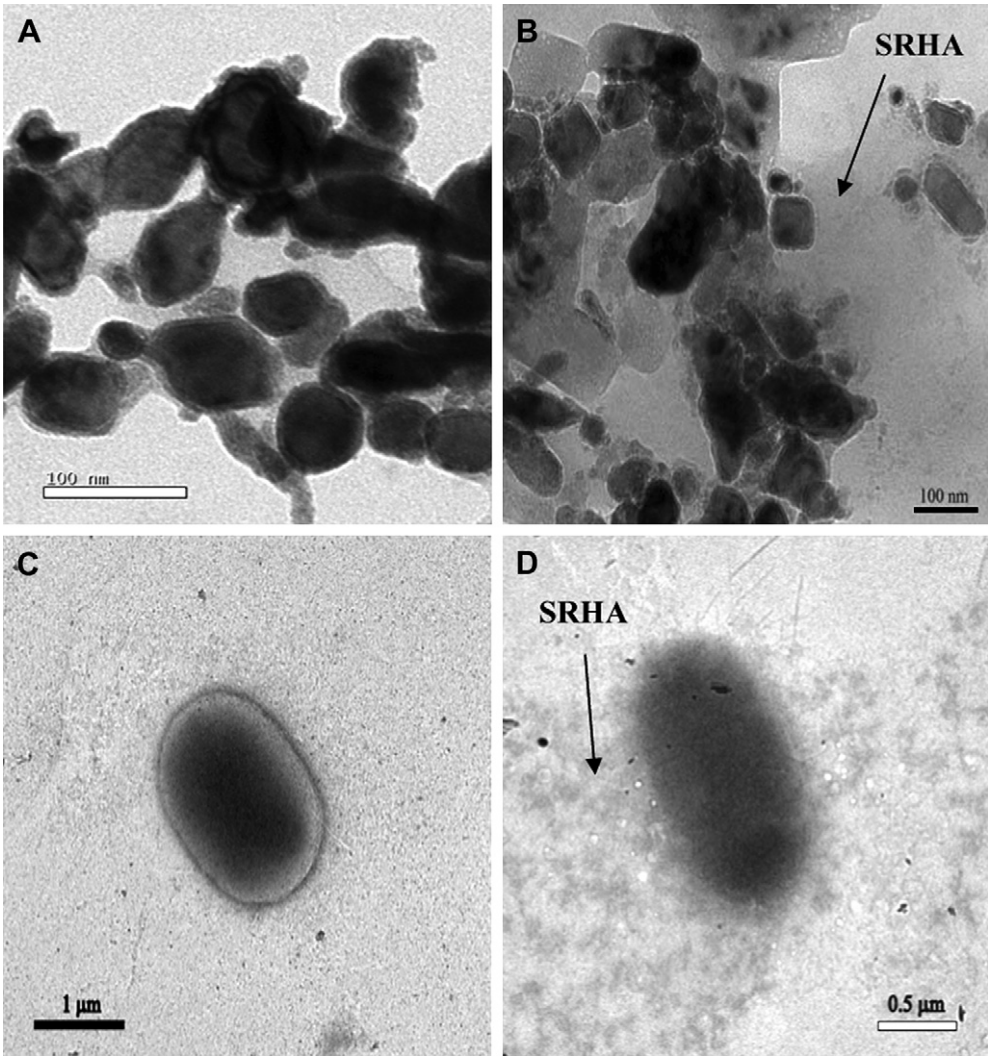

Fig. 3 – TEM images of NZVI and *E. coli* in the presence or absence of SRHA. (A) NZVI; (B) NZVI (1 g/L) in the presence of SRHA (10 mg/L); (C) *E. coli*; (D) *E. coli* in the presence of SRHA (10 mg/L).

both *E. coli* and *B. subtilis*. The solution ORP also remained constant (around −280 mV), indicating that changes in pH and ORP were not responsible for bacterial inactivation.

Bare NZVI partly aggregated and precipitated to the bottom of the reactor after 1 h incubation. In contrast, NZVI remained stable in the presence of SRHA (Fig. 2), indicating that SRHA associated with NZVI and changed its surface properties. This was corroborated by TEM observation (Fig. 3A,B).

SRHA decreased the zeta potential of NZVI from around −30 to −45 mV (Fig. 4). The zeta potential of bacterial cells also decreased in the presence of SRHA, from −26.9 ± 2.6 to −35.7 ± 1.7 mV for *E. coli*, and from −32.5 ± 1.2 to −45.6 ± 2.1 mV for *B. subtilis* after 1 h exposure, due to adsorption of SRHA onto bacterial cells (Fig. 3C,D).

3.3. *Reactivity of NZVI in the presence of SRHA*

Reductive dechlorination of TCE by NZVI followed first-order kinetics, and the degradation rate constant ($k_{obs-NZVI}$) decreased by 23% (from 0.0178 ± 0.0007 h^{-1} (R^2 = 0.99) to 0.0137 ± 0.0004 h^{-1} (R^2 = 0.99)) in the presence of SRHA (Fig. 5). This was likely due to occlusion of NZVI reactive sites (Redman et al., 2002). Note that SRHA did not significantly decrease the reactivity of NZVI with TCE during the first 4 h ($p > 0.05$; Fig. 5 insert), which indicates that toxicity mitigation over the 4 h exposure period was not due to a decrease in NZVI reactivity.

The generation of cathodic H_2, which is an indirect measure of NZVI corrosion and is also of interest due to its biostimulation potential (Till et al., 1998; Weathers et al., 1997) was also considered. H_2 production was not hindered by the presence of SRHA (Fig. 6), which actually resulted in a slight, statistically indiscernible enhancement. SRHA association with the surface of NZVI would reduce its direct contact with water, which is conducive to slower generation of H_2. However, this may be offset by SRHA-enhanced NZVI dispersion (Fig. 2), which is conducive to faster corrosion and H_2 generation.

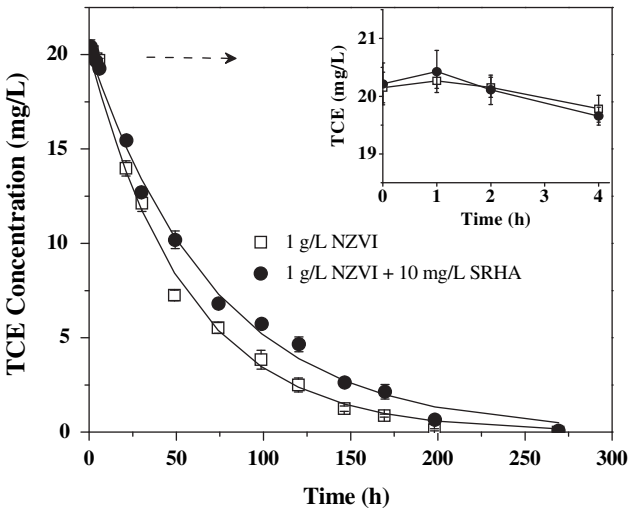

Fig. 5 – TCE degradation by NZVI in the presence and absence of SRHA. Degradation was first order with respect to TCE. Insert shows no effect of SRHA during the first 4 h.

3.4. *Mechanistic implications about toxicity*

The mechanism by which SRHA mitigates NZVI toxicity to bacteria cannot be fully discerned because the bactericidal mechanism has not yet been elucidated (Nel et al., 2009). Nevertheless, direct contact between NZVI and cells appears to play a critical role (Auffan et al., 2008; Lee et al., 2008; Xiu et al., 2010a). In this work, SRHA hindered direct contact by coating both NZVI and bacteria (Fig. 3). Although association with SRHA increased the (negative) surface charge of *E. coli* and NZVI (Fig. 4), both were also negatively charged in the absence of SRHA. Thus, electrostatic repulsion alone is unlikely to be the principal mechanism for NZVI toxicity mitigation. Partial or complete encapsulation of both NZVI and cells by a visible SRHA floccus apparently served as a physical barrier hindering direct contact. Accordingly, electrosteric repulsion was likely a greater hindrance to direct

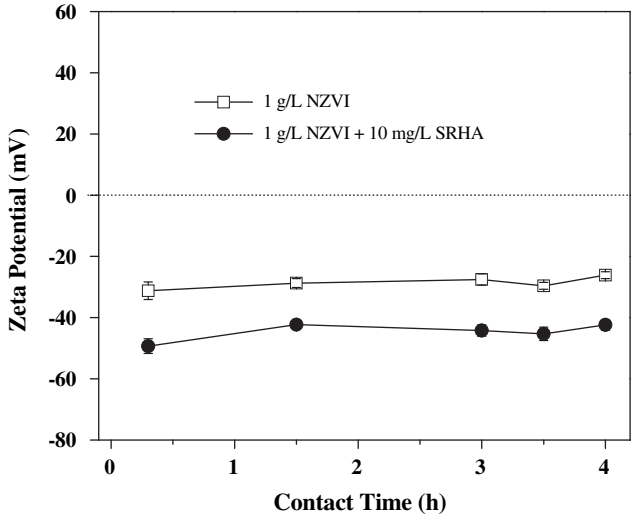

Fig. 4 – Zeta potential of NZVI in the presence or absence of SRHA in 2 mM bicarbonate solution.

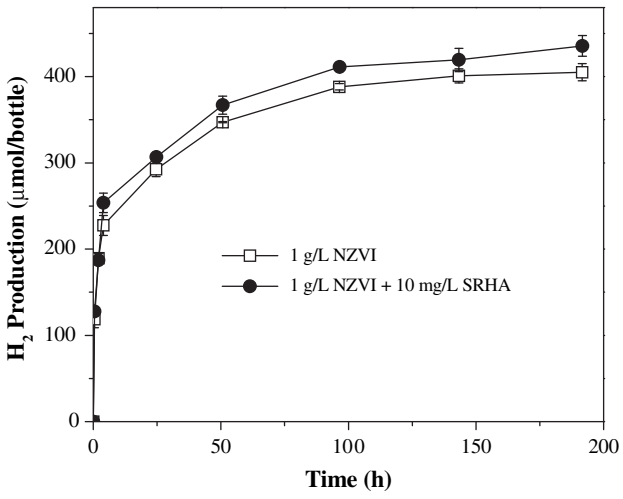

Fig. 6 – H_2 production by NZVI (1 g/L) with/without SRHA (10 mg/L) in bicarbonate solution.

bacterial contact than electrostatic repulsion alone, as observed previously for nZVI coated with synthetic polymers (Li et al., 2010). Coating by NOM has been observed for other nanoparticles (Baalousha et al., 2008; Diegoli et al., 2008; Hyung et al., 2007; Li et al., 2010) with an accompanying decrease in toxicity (Li et al., 2008). Therefore, electrosteric hindrance is likely the main mechanism by which humic acid mitigates NZVI toxicity (Phenrat et al., 2008, 2010). This mechanism helps explain why adding g/L concentrations of NZVI to contaminated aquifers does not significantly decrease biomass in those systems (e.g., Kirschling et al., 2010).

4. Conclusions

SRHA associated with both NZVI and cells and mitigated bacterial toxicity dramatically, likely due to the electrosteric hindrance of direct contact. Understanding the effect of NOM on cathodic H_2 evolution and TCE degradation is important because these are competing processes that consume Fe(0). Unchanged H_2 evolution with hindered TCE degradation in the presence of NOM suggests that greater amount of NZVI would be needed to treat a given TCE mass. However, H_2 evolution may also offer (as a positive tradeoff) a biostimulatory effect conducive to a higher potential for concurrent or sequential bioremediation of the target contaminants. Thus, further research may be warranted to optimize such biogeochemical interactions in groundwater remediation schemes.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.watres.2010.11.036.

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