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Adsorbed Polymer and NOM Limits Adhesion and Toxicity of Nano Scale Zerovalent Iron to *E. coli*

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Nanoscale zerovalent iron (NZVI) is used for groundwater remediation. Freshly synthesized bare, i.e. uncoated NZVI is bactericidal at low mg/L concentration, but the impact of surface modifiers and aging (partial oxidation) on its bactericidal properties have not been determined. Here we assess the effect that adsorbed synthetic polymers and natural organic matter (NOM) and aging (partial oxidation) have on the bactericidal properties of NZVI to the gram-negative bacterium, *Escherichia coli*. Exposure to 100 mg/L of bare NZVI with 28% Fe^0 content resulted in a 2.2-log inactivation after 10 min and a 5.2-log inactivation after 60 min. Adsorbed poly(styrene sulfonate) (PSS), poly(aspartate) (PAP), or NOM on NZVI with the same Fe^0 content significantly decreased its toxicity, causing less than 0.2-log inactivation after 60 min. TEM images and heteroaggregation studies indicate that bare NZVI adheres significantly to cells and that the adsorbed polyelectrolyte or NOM prevents adhesion, thereby decreasing NZVI toxicity. The 1.8-log inactivation observed for bare NZVI with 7% Fe^0 content was lower than the 5.2-log inactivation using NZVI with 28% Fe^0 after 1 h; however, the minimum inhibitory concentration (MIC) after 24 h was 5 mg/L regardless of Fe^0 content. The MIC of PSS, PAP, and NOM coated NZVI were much higher: 500 mg/L, 100 mg/L, and 100 mg/L, respectively. But the MIC was much lower than the typical injection concentration used in remediation (10 g/L). Complete oxidation of Fe^0 in NZVI under aerobic conditions eliminated its bactericidal effects. This study indicates that polyelectrolyte coatings and NOM will mitigate the toxicity of NZVI for exposure concentrations below 0.1 to 0.5 g/L depending on the coating and that aged NZVI without Fe^0 is relatively benign to bacteria.

Introduction

Nanoscale zerovalent iron (NZVI) is a highly redox active material used to treat heavy metal and chlorinated organic solvent contaminants found in groundwater and soil (*1*−*7*). NZVI reduces contaminants through the oxidation of the metallic iron core of the particle and subsequent transfer of electrons to the contaminant. The NZVI particles used in aquifer remediation will come in contact with subsurface bacteria, which can be important contributors to the remediation process for halogenated contaminants since they can assist in the degradation of PCE and TCE when zerovalent iron is present (*8, 9*). However, NZVI may adversely affect bacterial populations in the subsurface. Recent studies indicate that NZVI is bactericidal in pure cultures at concentrations as low as a few mg/L (*10, 11*), while NZVI is typically injected into the ground to achieve porewater concentrations in the grams per liter range (*2*). The bactericidal properties of NZVI are therefore important to understand as it may affect the remediation efficiency of NZVI if native bacterial populations are adversely affected. Further, significant toxicity of NZVI to native microbial communities may have other unintended ecological consequences (*12*).

NZVI is just one of many nanomaterials whose use has become widespread, with many potential applications. Silver (*13*), magnesium oxide (*14*), fullerenes (*15*), copper (*16*), titanium oxide (*17*), cerium oxide (*18*), and gold (*19*) have all been shown to display antimicrobial properties. The mechanism by which these nanomaterials cause cell death remains unclear, however, based on a recent summary of the biophysicochemical interactions of ∼130 nanoparticles at the nanobio interface (*20*), the mechanisms for toxicity may include disruption of the cell membrane structures (*21*), increased membrane permeability, interruption of energy transduction due to nanoparticle attachment to the membrane (*22, 23*), DNA and protein damage caused by released ions (e.g., silver *23, 24*), and apoptosis caused by the endogenous generation of reactive oxygen species (ROS) (*25*). Specific modes of action for the bactericidal properties of NZVI have been postulated to be reductive decomposition of protein functional groups in the cell membrane due to strong reducing conditions at the NZVI surface (*11*) and oxidative damage from intracellular ROS produced from Fenton's chemistry (*10, 11*).

Without cellular uptake, the oxidation state of iron atoms in NZVI and Fe-oxide nanoparticles have also been shown to correlate with its cytotoxicity to *E. coli* (*10*); NZVI (Fe(0)/Fe(II)/Fe(III)) being most toxic, magnetite (mixed Fe(II)/Fe(III) particle) less toxic, and maghemite (all Fe(III)) was nontoxic. NZVI exposed to environmental conditions will be oxidized under both oxic and anoxic conditions (*26, 27*). Oxidation of NZVI in groundwater can lead to the formation of different oxide layers, such as magnetite, maghemite, and lepidocrocite (*27, 28*). It is therefore possible that partially oxidized (aged) NZVI particles will be less toxic to *E. coli* than NZVI particles containing a larger amount of Fe^0 and therefore more redox active.

While NZVI toxicity may be mediated through ROS generation or through release of Fe^{2+} (*11*), it is reasonable to assume that close proximity of the NZVI to the bacteria would increase its toxicity potential. Prior reports of NZVI toxicity to *E. coli* have used uncoated NZVI which appears to have attached to the *E. coli* cell wall (*10, 11*). However, NZVI particles used in remediation are typically coated with polymer to increase its mobility in the subsurface (*29, 30*) or will become coated with natural organic matter (NOM) (*31*). Electrosteric repulsions caused by the negatively charged polymer coatings or adsorbed NOM may decrease the physical interaction between NZVI and the bacteria cell wall and therefore its toxicity. The effect of the NZVI coatings on its bactericidal properties has not been

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evaluated. The objectives of this study were to assess the effect that organic macromolecular coatings such as synthetic polymers and natural organic matter and aging (partial oxidation) have on the bactericidal properties of NZVI to a gram-negative bacterium, *Escherichia coli*.

Materials and Methods

Chemicals and NZVI. Sodium bicarbonate, hydrochloric acid, and agar were from Fisher Scientific (Howell, NJ). Poly(styrene sulfonate) (PSS, average MW = 70,000 g/mol) and humic acid (average MW = 1400 to 9200 g/mol (*32*)) were from Aldrich (St. Louis, MO). Miller LB broth was from Acros (Geel, Belgium). Polyaspartate (PA, average MW = 2500 g/mol) was from Lanxess (Pittsburgh, PA). All solutions and dilutions were prepared in distilled and deionized water (Barnstead Nanopure). Ultrahigh-purity N_2 was from Butler Gas products (Pittsburgh, PA). The NZVI, Reactive Nanoscale Iron Particles (RNIP 10-DS) and maleic acid co-olefin (average MW = 16,000 g/mol) modified RNIP (MRNIP2), was supplied by Toda Kogyo Corp. (Onoda, Japan). RNIP are redox reactive Fe^0/Fe_3O_4 core−shell NZVI particles. RNIP physical and chemical properties have been described previously (*5*). The Fe^0 content of the RNIP was determined by acid digestion and measurement of the H_2 formed, as previously described (*4*). The RNIP had been stored anaerobically in an aqueous slurry (pH = 12) for several months before and during the experimental program and had a Fe^0 content ranging from 20 to 28 wt % over the course of the study period.

NZVI Preparation. The procedure used to physisorb the polymers to the NZVI has been previously described (*33*). Briefly, a stock NZVI dispersion is diluted to a concentration of 120 g/L and ultrasonicated for 20 min. This dispersion is further diluted in a deoxygenated polymer solution to provide a final solution (30 mL) containing NZVI and polymer at a concentration of 3 g/L and 1 g/L, respectively, in a 70 mL bottle. Concentrated polymer suspension was always added to dispersed particles as order of operation was found to affect particle stability against aggregation. The 70 mL reactor was then rotated end over end for 24 h to reach adsorption equilibrium. This same method was also used to coat the particles with humic acid, with initial humic acid concentrations of 10 and 80 mg/L. After adsorption, the polymer coated NZVI suspensions were fractionated by allowing the most unstable particles to settle for 10 min. The stable suspension was decanted and used for exposure experiments after measuring the Fe^0 content by acid digestion. To evaluate the effect of Fe^0 content on toxicity to *E. coli*, the NZVI (28 wt % Fe^0) was aged in water to partially or fully oxidize the Fe^0 in the particle core. Two types of aged particles were used: anaerobically aged particles that contained 7 wt % Fe^0 and aerobically aged particles that contained no measurable Fe^0 as determined by acid digestion. Anaerobically aged particles were NZVI that had been aging in water in an anaerobic glovebox for 18 months. Aerobically aged particles were oxidized in O_2-saturated water for 24 h. After aging, polymer or NOM was physisorbed to the particles as described above.

***E. coli* Culture.** *E. coli* (ATCC strain 33876) was inoculated in 60 mL of LB Miller broth medium and grown at 37 °C for 12 h. The bacteria were harvested by centrifugation at 5000 g for 10 min. The supernatant was decanted, and the cells were resuspended in 5 mM sodium bicarbonate (pH = 8.2 ± 0.1) and then centrifuged again at 5000 g for 10 min. This washing step was repeated. The *E. coli* stock was prepared by resuspending the bacteria pellets in 15 mL of 5 mM sodium bicarbonate. The stock concentration of *E. coli* was determined to be in the range of 1×10^9 to 2×10^9 colony forming units (CFU) /mL by the spread plate method using LB agar plates incubated at 37 °C for 12 h.

Exposure Experiments and Minimal Inhibitory Concentration (MIC) Determination. The *E. coli* stock suspension was diluted 1000-fold in 5 mM bicarbonate solution in 20 mL culture tubes to reach final bacterial concentration of 10^6 CFU/mL. A suspension containing either bare, polymer-, or NOM-modified NZVI was added to the tubes to provide an initial NZVI concentration of 100 mg/L as determined by atomic absorption spectrometry (AAS). The culture tubes were placed on an orbital shaker at 350 rpm and 37 °C. Samples were taken at 0, 5, 15, 30, 45, and 60 min and serially diluted. The cells were plated in triplicate on LB agar plates and incubated for 12 h at 37 °C, and the colonies were counted.

Exposure under anaerobic conditions and aerobic conditions were conducted. For exposure under anaerobic conditions, the cell suspensions and the buffer solution were sparged with nitrogen gas for 30 min prior to adding NZVI and were capped to exclude oxygen. For aerobic exposure, the cell suspensions were not sparged, and the tubes were left open to the atmosphere to maintain oxygen saturation. Bacterial controls were performed side by side with the inoculation experiments. The controls consisted of cells (10^6 CFU/mL) in 5 mM sodium bicarbonate solution under both aerobic and anaerobic conditions to mirror the inoculation experiments.

The minimal inhibitory concentration (MIC) is defined as the lowest concentration of an agent that inhibits the visible growth of a microbe in an overnight culture (*34*). To measure the MIC of bare and coated NZVI 5 mL of the bacterial suspension was mixed with 5 mL of an NZVI suspension that ranged in concentration from 0.001 to 1 g/L in culture tubes. The MIC of free PSS in solution (a synthetic polymer that may have some toxicity) was also measured, with PSS concentrations ranging from 0.1 to 2 g/L. A 6.5 mL aliquot of the cell/NZVI or polymer suspension was added to 6.5 mL of LB Miller broth and incubated at 37 °C for 12 h. The tube with the lowest concentration of NZVI to remain unclouded was recorded as the MIC (*35*).

Measuring the Dispersed Iron Concentration in Culture Tubes. The uncoated NZVI used in this study aggregates rapidly (*36*). The coated NZVI will also agglomerate to some degree and partially sediment from solution despite the coating applied (*33*). NZVI aggregation was monitored by measuring the concentration of suspended particles over time during quiescent settling in 5 mM bicarbonate solution using UV−vis (λ = 508 nm) as previously described (*33*). Attachment of NZVI to the *E. coli* could prevent NZVI aggregation and keep it suspended in solution. The UV−vis method did not work with cells present due to scattering interference from the cells, so to monitor heteroaggregation between NZVI and the cells the mass of bare or modified NZVI that remained in suspension after mixing with the cells was measured. A 5 mL aliquot was removed from the supernatant and acid digested in concentrated HCl to dissolve the suspended NZVI. The samples were then analyzed by AAS to determine the amount of iron that remained in suspension. Control samples without *E. coli* were measured in the same way to determine the amount of NZVI that remained in suspension due to sorption to the cell membrane.

Transmission Electron Microscopy (TEM). The treated and untreated *E. coli* cells were fixed using 1% formaldehyde and 1% glutaraldehyde in 5 mM sodium bicarbonate buffer for 24 h at 4 °C and washed three times with 5 mM sodium bicarbonate buffer. A 1 μL aliquot of the fixed cells was then put on a carbon coated copper grids for TEM examination. Cells were exposed to 10 mg/L of bare or coated (MRNIP2) NZVI.

Results and Discussion

NZVI Cytotoxicity Assessment. Exposure to 100 ppm of uncoated NZVI having 20% Fe^0 under anaerobic conditions resulted in a 2.2-log reduction in viable *E. coli* cells after 10

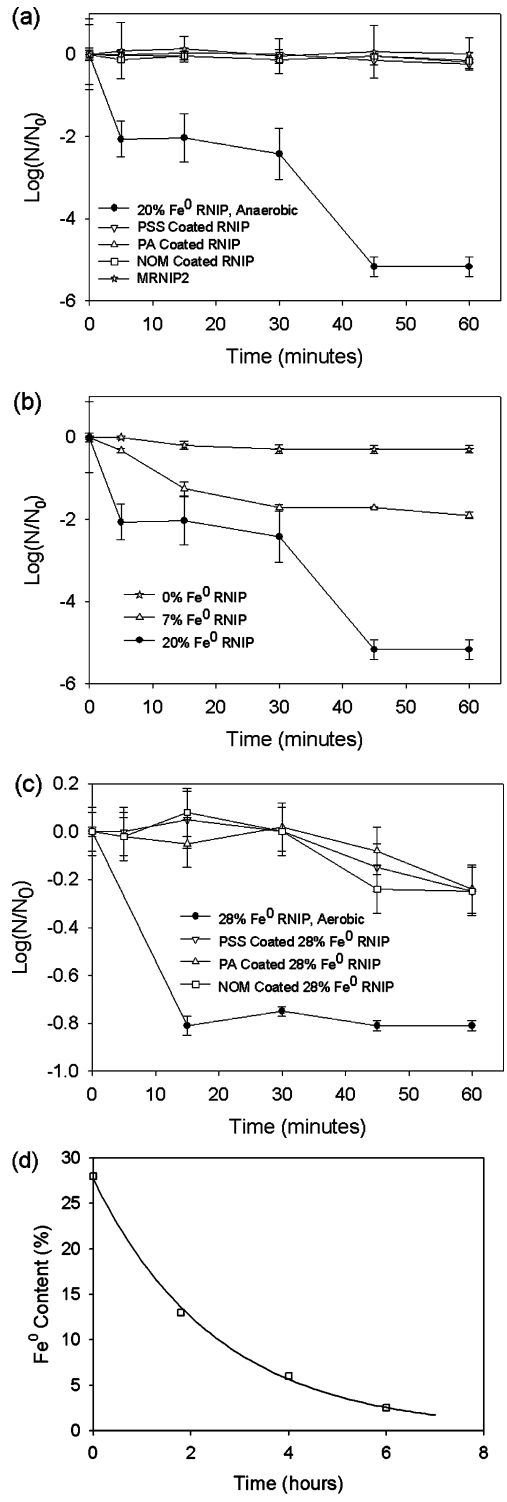

FIGURE 1. (a) Exposure of *E. coli* to bare NZVI and coated NZVI under anaerobic conditions; (b) exposure of *E. coli* to aged NZVI, containing 0%, 7%, and 20% Fe^0 under anaerobic conditions; (c) exposure of *E. coli* to NZVI and coated NZVI under aerobic conditions; and (d) Fe^0 content in NZVI as a function of exposure time to air-saturated water (NZVI concentration was approximately 1 g/L). Oxygen was continuously bubbled through the vessel during aging.

min and up to a 5.2-log reduction in viable *E. coli* cells after 1 h (Figure 1a). This is consistent with previous reports of NZVI toxicity; 3.4- and 4.5-log reductions in viable *E. coli* cells were observed after 10 min and 1 h exposure to 9 ppm NZVI, respectively. However, the NZVI used previously appears to exert toxicity toward *E. coli* more quickly than RNIP (3.4-log inactivation after 10 min (*11*) vs 2.5-log inactivation after 10 min in this study). One explanation for the apparent increase in toxicity is that the NZVI used in previous studies was produced by borohydride reduction of dissolved iron (Fe(B)) (*11*), whereas the NZVI used here (RNIP) was made from reduction of Fe-oxides by H_2 gas. The most relevant differences between Fe(B) and RNIP are their Fe^0 content, Fe(B) is upward of 98 wt % Fe^0 compared to 20−28 wt % Fe^0 for RNIP, and the amount of Fe(II) released during reaction, Fe(B) undergoes oxidative dissolution with dissolved Fe(II) accounting for as much as 40% of the NZVI at pH = 8 whereas the Fe_3O_4 shell of RNIP limits Fe(II) solubility at pH = 8 (*5*). Both Fe^0 content and the presence of Fe(II) have been positively correlated with NZVI toxicity to *E. coli* (*10, 11*). Further, Fe(B) has been shown to generate ROS such as hydroxyl radicals that may be toxic to *E. coli* (*37*). Thus Fe(B) has more Fe^0, produces more Fe(II), and can produce ROS which may inactivate the cells faster than RNIP (*11*). The primary particle size and N_2−BET specific surface area of Fe(B) and RNIP are similar so these properties are not likely to be responsible for the differential toxicity observed. Despite the physicochemical differences between RNIP and Fe(B), however, the total inactivation of *E. coli* after 1 h was similar in both cases suggesting that the mode of action of toxicity is related to the presence of Fe^0 and the particle's overall redox activity rather than external factors such as Fe(II), size, or surface area. It also suggests that physical proximity of the NZVI to the cell is needed to exert toxicity; i.e., not through a soluble carrier like Fe(II).

Effect of Coatings. Exposure of the same NZVI particles coated with PSS, PA, maleic acid co-olefin, or NOM resulted in less than a 0.2-log reduction in viable *E. coli* cells, while a 5.2-log reduction was observed for bare NZVI. All of these coatings reduced the cytotoxicity of NZVI to *E. coli*; however, the mechanism by which coatings inhibit toxicity is not clear. One explanation is that coatings prevent the physical contact between NZVI and cells. The cellular uptake and toxicity mechanisms proposed by Lee et al., ROS produced from intracellular oxygen and reductive decomposition of functional groups in the proteins and lipopolysaccharides of the outer membranes (*11*), would require close contact between NZVI and cells. Additionally, previous studies have shown that contact between cells and nanoparticles is needed to cause cell death for other nanoparticles: Quantum dots that have their coatings weathered show an increase in toxicity (*38*); adsorbed natural organic matter reduces the toxicity of fullerenes by reducing direct contact with cells (*39*); and carbon nanotubes that have physicochemical properties that enhance cell-nanotube contact have a higher toxicity (*40*). TEM images of *E. coli* exposed to 10 mg/L bare and coated NZVI appear to support the hypothesis that the NZVI surface coatings prevent contact between the NZVI and the cells (Figure 2). Figure 2a shows that bare NZVI readily attaches to the cell membrane. Bare Fe(B) used in previous studies also showed adhesion of the NZVI to the cell walls (*10, 11*). However, the coated particles do not appear to attach to *E. coli* (Figure 2b). This observation was true for all *E. coli* observed on the grids (>20).

To further investigate attachment of bare and coated NZVI to *E. coli* cells, the aggregation and sedimentation of bare and coated NZVI was measured in the absence and in the presence of *E. coli* (10^6 CFU/mL). Despite the polymer and NOM coatings on NZVI, in all cases a fraction of the NZVI (100 mg/L) was not stable in 5 mM $NaHCO_3$ buffer and sedimented rapidly from solution (Figure S1, Supporting Information). In particular, bare NZVI rapidly aggregates and sediments from solution, as has been previously reported (*36*). After each 60 min exposure period, the particles were allowed to settle quiescently, and a 5 mL sample of the supernatant was removed and analyzed for total iron content.

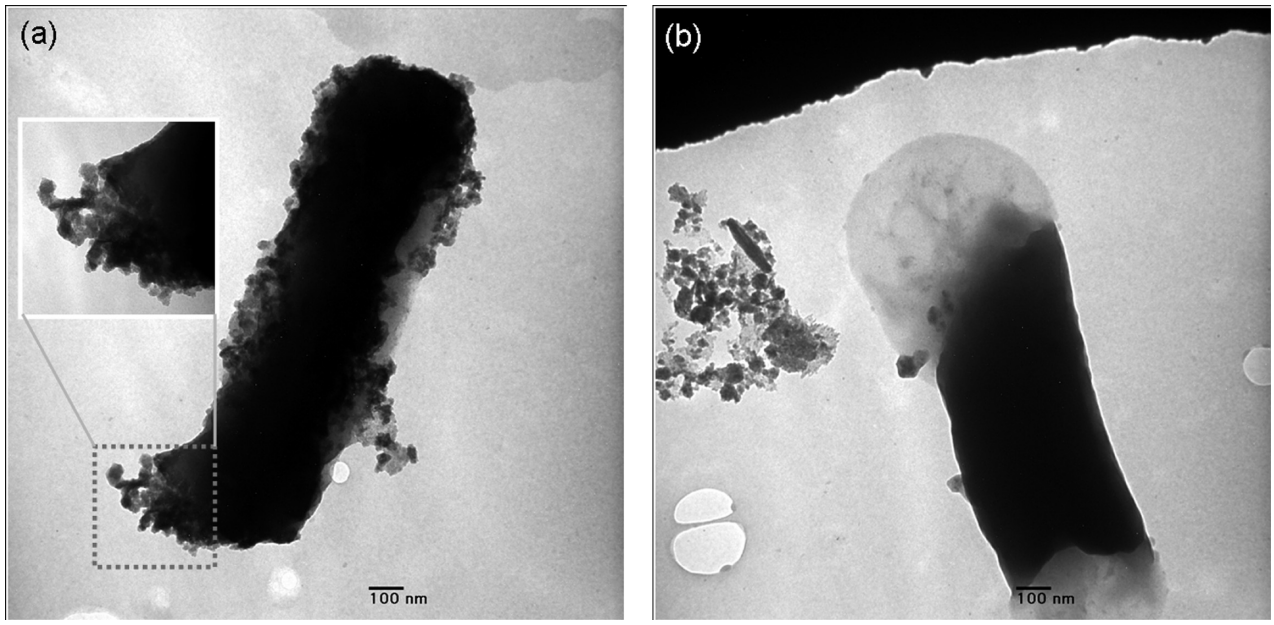

FIGURE 2. TEM images of *E. coli* incubated with (a) 10 mg/L of bare NZVI (20% Fe^0) and (b) 10 mg/L of MRNIP2 (coated with olefin maleic acid, MW = 16, 000 g/mol) for 1 h. The globular material on the cells was observed for all cells that had been exposed to MRNIP2. The chemical composition of the material is not known, but it did not appear to affect cell viability.

TABLE 1. MIC and the Change in Suspended Iron after Contact with Bacteria Cells

		% change* of iron remaining in suspension after contact with *E. coli*	
iron	**MIC (mg/L)**	**anaerobic**	**aerobic**
28% Fe^0 NZVI	5	50	250
7% Fe^0 NZVI	5	80	170
PSS NZVI	516	−20	no change
PA NZVI	140	no change	−10
NOM NZVI	100	−20	no change
PSS polymer	<2 g/L	no change	no change

* All values are ±5%.

The iron suspended in the presence of cells was compared with the iron suspended in identical reactors but without the cells. The percent increase (+) or decrease (-) in suspended iron due to the presence of cells under both anaerobic and aerobic exposures are provided in Table 1. For bare NZVI under either aerobic or anaerobic conditions, the presence of *E. coli* resulted in 50 to 250% more NZVI remaining in suspension compared to the absence of cells. The increase in suspended NZVI in the presence of cells under aerobic conditions compared to anaerobic conditions is unclear. The amount of polymer and NOM coated NZVI remaining in suspension did not change or was slightly lower in the presence of cells than without. This indicates that bare NZVI is physically interacting with and attaching to the cells, whereas NZVI with physisorbed synthetic polymer or NOM are not physically attaching to *E. coli*. This observation is consistent with the TEM micrographs (Figure 2), which also indicate adhesion of bare NZVI to cells and limited adhesion of coated NZVI to cells.

The polyelectrolyte and NOM coatings on NZVI can decrease adhesion by several different methods: electrostatic, steric, and electrosteric repulsion (Figure 3). Low MW adsorbed polyelectrolytes that do not extend or form brushes provide electrostatic repulsions. Large uncharged polymers can afford steric repulsions by inhibiting close contact between the particles and the cells. Large MW polyelectrolytes afford both an electrostatic and steric repulsion, known as electrosteric repulsion, that is stronger than either electrostatic repulsion or steric repulsion (*33*), and its repulsive force is less sensitive to changes in ionic strength or pH than electrostatic repulsions that would occur using bare (uncoated) particles. NOM adsorbed to the particles decreased NZVI toxicity similarly as the polyelectrolytes tested here. NOM is probably acting as a polyelectrolyte to provide electrosteric repulsions between the particles and the cells. Polyelectrolytes such as poly(maleic acid) have been used as NOM analogs because they have the same types of functional groups and behave similarly to NOM in natural waters (*41*).

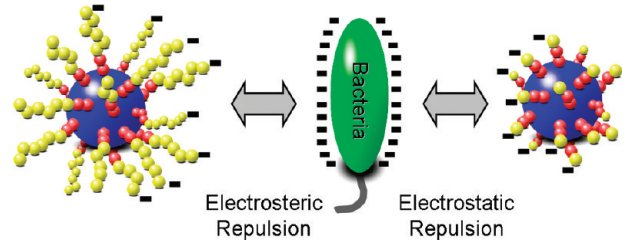

FIGURE 3. The surface coatings on NZVI may prevent physical interaction with *E. coli*. Higher MW polyelectrolyte coatings provide electrosteric repulsive forces, while low MW surfactants and polyelectrolytes provide predominantly electrostatic repulsive forces that are more prone to charge screening with increasing ionic strength. Uncharged polymers provide steric repulsions (not shown). Natural organic matter can act as a polyelectrolyte or surfactant, providing electrostatic or electrosteric repulsive forces.

Effect of Fe^0 Content. Auffan et al. showed that iron nanoparticles containing either Fe(II) or Fe^0 are more cytotoxic to *E. coli* than particles with only Fe(III) (*10*). To determine if toxicity was a function of the iron zero content, *E. coli* toxicity from exposure to NZVI with 7% Fe^0 were compared to those with 20% Fe^0 (Figure 1b). The Fe^0/Fe_3O_4 core shell structure of NZVI is preserved during anaerobic oxidation by water so the more oxidized particles are identical except with a thicker magnetite layer and less Fe^0 (*5*). The other type of particle that was tested was aged aerobically and contained no Fe^0. In the presence of dissolved oxygen (8.9 mg/L) the Fe^0 content of the particles is very rapidly consumed (Figure 1d), and the oxide that is formed is likely a combination of magnetite, maghemite, and possibly

lepidocrocite (*26*−*28*). Particles with no Fe^0 remaining had little to no effect on *E. coli* cells (less than 0.2-log reduction in cells), while particles containing 7% Fe^0 caused a 2-log reduction in cells (Figure 1b). This is less than the 5.2-log reduction caused by particles with 20% Fe^0. This indicates that percentage of Fe^0 is related to NZVI cytotoxicity, consistent with previous reports (*10*). These results suggest that as NZVI ages anaerobically, its toxicity to bacteria will decrease. When aged anaerobically, the half-life time of Fe^0 in this particular type of NZVI ranges from several weeks to several months depending on the pH of the groundwater and the contaminant loading (*42*), so time scales for this toxicity reduction should be months to just a few years after injection without dissolved oxygen present. With dissolved oxygen present, the Fe^0 lifetime is very short (~2 h) (Figure 1d), and NZVI should rapidly oxidize to become nontoxic.

Effect of Aerobic and Anaerobic Conditions. NZVI (28% Fe^0 content) was less toxic to *E. coli* exposed under aerobic conditions (0.8-log inactivation) compared to exposure under anaerobic conditions (5.2-log inactivation) (Figure 1c). NZVI particles are quickly oxidized under aerobic (Figure 1d). The reason for the decreased toxicity is, however, probably not the loss of Fe^0 in the particles during exposure under aerobic conditions. The toxic effects on *E. coli* also appear to be mitigated after 15 min of exposure (Figure 1c), whereas the Fe^0 content is not depleted significantly during this time (Figure 1d). The lower observed toxicity during exposure under aerobic conditions could be a result of the formation of a different type of Fe-oxide at the particle surface, such as maghemite (*26, 28*), when aged aerobically compared to aging under anaerobic conditions. This different Fe-oxide may decrease adhesion to the cells, inhibit electron transfer due to the formation of Fe-oxides on the particle surface that are less conductive than magnetite, or lower the amount of Fe(II) adsorbed to or within in the surface oxide (*26*) and therefore its toxicity to *E. coli*. It is also possible that under aerobic conditions, *E. coli* express enzymes that destroy ROS such as superoxide dismutase. These hypotheses remain to be evaluated. Finally, it may be possible that under aerobic conditions, the smallest NZVI particles and the ones present in the greatest number are fully oxidized, rendering them nontoxic to *E. coli*. It has been previously shown that the smallest size fraction of NZVI in a polydisperse dispersion of RNIP contained only 4 wt % Fe^0, whereas the larger size fraction contained 63% Fe^0 (*43*).

Minimum Inhibitory Concentrations (MIC). The minimum inhibitory concentrations were measured for bare and coated NZVI to confirm the plate count studies and to further distinguish differences in the antimicrobial properties of the various coating types (Table 1). The MIC for bare NZVI was 5 mg/L using either 7 or 28% Fe^0 content. Thus, after 24 h, NZVI with Fe^0 remaining in the particles will exhibit some toxicity. This MIC is significantly higher than those measured for silver nanoparticles (13 μg/L (*44*)) and slightly higher than those measured for bare and poly(vinylpyrrolidone) nC_{60} fullerene aggregates (*45*), indicating that NZVI toxicity potential is lower than for these other manufactured nanomaterials.

The measured MICs for the NOM-, PSS-, and PAP-coated NZVI are 20 to 100 times greater (i.e., less toxic) than for uncoated particles. The longer term (24 h) MIC results further confirm the short-term (1 h) plate count results, which showed that coated NZVI was nontoxic or considerably less toxic than the bare NZVI. No inhibition was observed for just the PSS polymer solution in the concentration range that was used. The order of toxicity measured by the MIC test was PSS < PAP < NOM. This follows the order of the ability of the surface coating to stabilize the particles against aggregation at short times (<20 min) (Figure S1, Supporting Information) as previously reported (*33*). The adsorbed mass and adsorbed layer thickness of PSS, PAP, and NOM on NZVI have been reported (*33, 46*). The adsorbed mass was similar for NOM (0.4 to 2.2 mg/m^2) and PSS and PAP (1.9 to 2.2 mg/m^2), but the adsorbed layer conformation and thickness were different. The thicker polymer brush afforded by PSS (67 ± 7 nm) does a better job of preventing aggregation than do adsorbed PAP (40 ± 12 nm) or NOM (4 ± 3 nm) which adsorb with a flatter conformation on the NZVI surface. The same forces that prevent aggregation will also prevent adhesion to bacteria, so the order of toxicity measured by the MIC test is consistent with the hypothesis that surface coatings that best prevent adhesion of NZVI to the cells have the lowest toxicity.

All of the MIC values were less than 10 g/L, a typical concentration that NZVI is injected into groundwater (*2*). This suggests that inhibition of microbial activities may occur in the vicinity of the injection point, even for coated NZVI. Adsorbed NOM significantly decreases the measured NZVI toxicity to *E. coli*. NOM is ubiquitous in the environment, and all NPs will ultimately become coated with NOM. While fullerenes (nC_{60}) have been shown to be highly toxic to bacteria in the absence of environmental media, fullerene had no effect on a soil microbial community, even at concentrations as high as 10,000 ppm (*47*). This study indicates that one reason for the lack of ecological effects may be adsorbed NOM derived from soil. Similarly, adsorbed NOM completely eliminated nC_{60} antimicrobial activity (*39*) and decreased the antimicrobial activity of PVP-coated Ag nanoparticles to *P. fluorescens* (*48*). The results presented here are consistent with these observations and indicate that the most likely reason for the decreased toxicity in the presence of NOM or adsorbed polyelectrolyte is that the electrosteric repulsions afforded by the adsorbed polyelectrolyte are limiting adhesion to the bacteria. This suggests that the bactericidal effects of manufactured nanoparticles can, at least in part, be mitigated through the proper selection of surface coatings. However, in the case of NZVI, the adsorbed coatings decrease reactivity with the target contaminant (*49*). Thus, decreasing toxicity through surface modification will likely involve trade-offs with performance.

Acknowledgments

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Supporting Information Available

Aggregation and sedimentation of bare and polymer coated NZVI particles. This material is available free of charge via the Internet at http://pubs.acs.org.

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