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Nanoscale zerovalent iron alters soil bacterial community structure and inhibits chloroaromatic biodegradation potential in Aroclor 1242-contaminated soil

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ABSTRACT

Nanoscale zerovalent iron (nZVI) has potential for the remediation of organochlorine-contaminated environments. Environmental safety concerns associated with in situ deployment of nZVI include potential negative impacts on indigenous microbes whose biodegradative functions could contribute to contaminant remediation. With respect to a two-step polychlorinated biphenyl remediation scenario comprising nZVI dechlorination followed by aerobic biodegradation, we examined the effect of polyacrylic acid (PAA)-coated nZVI (mean diameter = 12.5 nm) applied at 10 g nZVI kg^{-1} to Aroclor-1242 contaminated and uncontaminated soil over 28 days. nZVI had a limited effect on Aroclor congener profiles, but, either directly or indirectly via changes to soil physico-chemical conditions (pH, Eh), nZVI addition caused perturbation to soil bacterial community composition, and reduced the activity of chloroaromatic mineralizing microorganisms. We conclude that nZVI addition has the potential to inhibit microbial functions that could be important for PCB remediation strategies combining nZVI treatment and biodegradation.

1. Introduction

Reducing the size of particles to the nanoscale (<100 nm) frequently results in physico-chemical properties and reactivities that differ from those of larger scale particles with the same chemical composition (Handy et al., 2008). Within environmental remediation, the increased reactivity of nanoscale zerovalent iron (nZVI) together with the enhanced potential for mobility is of particular interest with regard to the treatment of nitrate, chlorinated solvent, organochlorine pesticide and polychlorinated biphenyl contaminated environments (Wang and Zhang, 1997; Zhang and Elliott, 2006; Zhang, 2003).

The deployment of nanoscale materials to the environment as in situ remediation technologies has raised concerns about their environmental safety (Auffan et al., 2009; Handy et al., 2008). Under in vitro conditions nZVI has been shown to be bactericidal to *Escherichia coli* and *Bacillus nealsonii* in aqueous cultures (Auffan et al., 2008; Fajardo et al., 2012; Lee et al., 2008; Li et al., 2010) and although nZVI will be deployed to chemically contaminated (and therefore potentially containing structurally perturbed microbial communities) environments, it is important that the nZVI does not impact negatively on indigenous microbial communities with functions relevant to contaminant biodegradation (as a parallel or subsequent remedial process). In environmental matrices there is the potential for the antimicrobial properties of nZVI to be modulated through interaction with matrix components (Chen et al., 2011; Li et al., 2010), yet there are only a few studies in which nZVI effects on indigenous microbes and their activities have been examined (Barnes et al., 2010a, 2010b; Cullen et al., 2011; Fajardo et al., 2012; Kirschling et al., 2010). Furthermore, in the limited number of studies that have examined nZVI interactions with indigenous microbial communities with biodegradation potential, the underlying remediation scenario has been trichloroethylene contaminated groundwater or saturated aquifer material (Barnes et al., 2010a; Kirschling et al., 2010).

Although nZVI has been most frequently deployed for remediation of the saturated sub-surface, there is increasing interest in its potential for soil remediation (Naja et al., 2009; Satapanajaru et al., 2008). Therefore, our overall aim here was to provide novel information regarding microbial impacts of nZVI when applied as a remedial treatment by focus on unsaturated surface soil. Our experiments, as described below, comprise a polychlorinated biphenyl (PCB) remediation scenario where nZVI treatment is combined with aerobic bioremediation.

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PCBs are persistent organic pollutants, the production and use of which has been targeted for elimination under the Stockholm Convention on Persistent Organic Pollutants and it is estimated that 99% of the environmental PCB burden resides in soils (Travis and Hester, 1991). Ex situ technologies such as incineration, thermal desorption and soil washing have been the most popular means of remediating PCB-contaminated soil (Dàvila et al., 1993), however, the efficacy of microbial degradation for the in situ bioremediation of PCB-contaminated soil has also frequently been assessed (Abraham et al., 2002; Pieper and Seeger, 2008). Typically, under aerobic conditions microorganisms can relatively easily transform less chlorinated PCB congeners to corresponding chlorobenzoates that can then be further transformed and mineralized (Pieper and Seeger, 2008). Highly chlorinated congeners, however, are generally recalcitrant to aerobic transformation although they can undergo reductive dechlorination under anaerobic conditions to produce less chlorinated congeners (Abraham et al., 2002). Abiotic nZVI-mediated transformation of chlorinated organics is also effected by reductive dechlorination yielding less chlorinated or unchlorinated products (Matheson and Tratnyek, 1994). As nZVI has been shown to be active in the partial dechlorination of larger molecular weight PCBs in mixtures of water and alcohol (Lowry and Johnson, 2004; Wang and Zhang, 1997), its application in conjunction with appropriate stabilizers such as carboxymethyl cellulose or polyacrylic acid (PAA) to reduce agglomeration (Naja et al., 2009; Satapanajaru et al., 2008; Yang et al., 2008), has the potential for use in a two-step in situ soil remediation approach comprising nZVI reduction followed by aerobic biodegradation.

Using a set of laboratory-incubated soil microcosms, artificially contaminated with the PCB Aroclor-1242, we therefore investigated the impacts of PAA-coated nZVI on microbial abundance, biodegradative activity and community composition over a period of 28 days. As PAA has previously been shown to alter the availability of substrates and metal co-factors (Guiwei et al., 2008), we also investigated its microbial impact. For assessment of soil biodegradative populations, we chose 2,4-dichlorophenoxyacetic acid (2,4-D) as a model chlorinated mono-aromatic due to: 1) the convergence (at chlorocatechol) of its biodegradation pathway with that of 3-chlorobenzoate (Perez-Pantoja et al., 2008), a key intermediate in PCB biodegradation; and 2) its availability in ^{14}C-labelled form, enabling quantification of its mineralization to CO_2 against a complex background of other C sources. We hypothesized that 1) nZVI will have a greater effect on soil microbial populations than PAA stabilizing solution, 2) the nature and extent of the impact will depend on soil Aroclor contamination.

2. Materials and methods

2.1. Soil and nanoparticles

Rowland series soil (5–20 cm depth) was collected from an arable field on the University of Reading farm (U.K. Grid Reference SU763753) and sieved to 2 mm in the field moist state. Soil was stored at 4 °C for two weeks before use. Zerovalent iron nanoparticles (mean particle diameter = 12.5 ± 0.3 nm) were supplied by Golder Associates Inc. (New Jersey, U.S.A.) as an aqueous stock solution of nZVI in 0.18% (w/v) polyacrylic acid (PAA; average Mw ~1200; density = 1.32 g mL^{-1}, Aldrich, St. Louis, U.S.A.) dispersant. A description of the physico-chemical properties of the soil and nanoparticles and their handling has been previously published (Cullen et al., 2011). At the time of use, the concentration of nZVI particles in suspension was 506 ± 10.5 g L^{-1} (determined gravimetrically for four aliquots dried at 105 °C) and the suspension was shaken vigorously prior to use.

2.2. Time-course experiment

A 28-day time-course experiment was established with the following factors: Aroclor-1242 contamination (0 or 50 mg kg^{-1} dry weight soil) and soil amendment (nZVI in PAA dispersant, PAA only or water only) resulting in six treatments in total that were applied to 4 g (dry weight basis) aliquots of soil in 40 mL glass EPA vials. Vials receiving Aroclor-1242 (Supelco, Bellefonte, U.S.A.) were spiked with a 500 μg mL^{-1} stock solution in hexane to give the required initial concentration of 50 mg Aroclor kg^{-1} dry weight soil. The soils were then placed in a fume cupboard for 1 h to allow the hexane to evaporate. Uncontaminated soils were treated similarly using only hexane. The nZVI particles were applied as aqueous dilutions for simultaneous supply of 10 g nanoparticles kg^{-1} soil (equivalent to field application for remediation purposes, Personal Communication, M.J. Borda, Golder Associates) and adjustment of soil water content to 60% water holding capacity. Distilled water, or an aqueous dilution of 45% (w/w) polyacrylic acid (PAA), sodium salt (average M_w ~1200) to supply 35.6 mg PAA kg^{-1} soil (equivalent to that supplied in the nZVI slurry) were added to the other treatments as appropriate. Solutions were distributed throughout the soil by vortexing for 10 s. After 0, 1, 4, 7 and 28 days' of incubation at 25 °C in the dark, replicates ($n = 4$) were destructively sampled for analysis of soil physico-chemical properties, PCB concentration, the size and activity of the chloroaromatic mineralizing microbial population and bacterial community composition as described below.

2.3. Soil physico-chemical properties

Redox potentials were measured in undisturbed soil using a platinum redox probe (Bibby Scientific, Dunmow, U.K.) and are referred to the hydrogen scale (Cullen et al., 2011). Soil pH was measured in distilled water (2:5 w/v suspensions). Nitrite and nitrate concentrations in 1 M KCl extractions (2:5 w/v) were determined colorimetrically at 520 nm (Grimshaw et al., 1989) using a ChemLab System 4 autoanalyzer (ChemLab Instruments, Hornchurch, U.K.).

2.4. PCB concentrations

PCBs were extracted from soil using a 1:1 v/v mixture of acetone and hexane after U.S. Environmental Protection Agency Method 8082A (USEPA, 2007). GLC – pesticide residue grade (Fisher Scientific, Loughborough, U.K.) solvents were used and 1.5 mg L^{-1} decachlorobiphenyl (Supelco, Bellefonte, U.S.A.) was included as an internal standard. PCBs were partitioned into the hexane component by the addition of 4 mL distilled water. After 15 min the hexane layer was transferred to GC vials and stored at −20 °C until analysis by gas chromatography using an Agilent 6890N gas chromatograph fitted with a micro electron-capture detector (μECD) and a 30 m × 320 μm × 0.25 μm film 5% diphenyl, 95% dimethyl siloxane capillary column (Supelco, Bellefonte, U.S.A.). A 2 μL splitless injection was injected via an autosampler and subjected to an initial temperature of 100 °C for 2 min, ramped up to 160 °C at 15 °C min^{-1} and again to 270 °C at 5 °C min^{-1}, and held at 270 °C for 1 min with a constant flow rate of helium carrier gas of 1.1 mL min^{-1} (USEPA, 2007). Out of the 38 peaks present in the soil spiking solution, 35 peaks showed a linear response on dilution and their peak areas were summed to indicate the concentration of Aroclor-1242 present in soil. Extraction efficiency was in excess of 90% for all soil treatments.

2.5. Size and activity of the chloroaromatic-mineralizing microbial population

The herbicide 2,4-D was used as a model chlorinated mono-aromatic in both cases. The size of the chloroaromatic-mineralizing microbial population was estimated using a ^{14}C-based most probable number technique (Shaw and Burns, 2004). Briefly, five replicate 0.1 g (dry weight) soil sub-samples were suspended in 1.0 mL basal medium. This 10^{-1} dilution was used to create further ten-fold dilutions ranging between 10^{-2} and 10^{-5} in 6 mL polyethylene vials. Each vial was supplemented with [^{14}C]-*UL*-2,4-D (Supelco, Bellefonte, U.S.A.) to give a final concentration of 50 μg mL^{-1} 2,4-D and an activity of 8 Bq mL^{-1}. After 6 weeks' incubation at 20 °C, the remaining radioactivity was quantified using a Beckman LS 1801 liquid scintillation counter (Beckman, Irvine, U.S.A.). UltimaGold (PerkinElmer, Groningen, Netherlands) was used as the scintillant in a 1:4 sample-to-cocktail ratio. Tubes with <35% of the radioactivity of uninoculated tubes were considered to demonstrate 2,4-D mineralization and were scored positive. $MPN_{2,4\text{-}D}$ estimates were derived by reference to MPN tables (Alexander, 1982). There was no evidence of nZVI particles carried over from amended soil inhibiting 2,4-D mineralization in the assay tubes and consequently giving rise to false negative MPN results. The activity of the chloroaromatic-mineralizing population was determined through spiking soil with [^{14}C]-*UL*-2,4-D (50 μg 2,4-D g^{-1} soil, 250 Bq g^{-1} radioactivity) followed by alkaline trapping and quantification of mineralized ^{14}C–CO_2 for 30 days (Shaw and Burns, 2004). The ^{14}C–CO_2 traps (1 M KOH) were replaced every 3–4 days and the activity of the KOH removed was determined by liquid scintillation counting as described above.

2.6. Bacterial community composition

Soil DNA was extracted, amplified by polymerase chain reaction (PCR) and subjected to denaturing gradient gel electrophoresis (DGGE) as described in Watkins et al. (2009). The purity and concentration of the extracted DNA was determined using a Nano Drop 2000 spectrophotometer (Thermo Scientific, Wilmington, U.S.A.). The 16S ribosomal RNA gene in 100 ng DNA was amplified by PCR using the 357f-GC (forward) and 518r (reverse) primers of Muyzer et al. (1993). The thermocycler program was a 1 min denaturation phase at 95 °C followed by 32 cycles of