International Biodeterioration & Biodegradation 127 (2018) 241–246

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Interactions of nanoscale zero valent iron and iron reducing bacteria in remediation of trichloroethene

Lenka Honetschlägerová[a,*], Radek Škarohlíd[a], Marek Martinec[a], Marek Šír[a], Vittorio Luciano[b]

[a] Department of Environmental Chemistry, University of Chemistry and Technology Prague, Technická 5, 16628, Prague, Czech Republic
[b] Department of Land, Territory and Infrastructures Engineering, Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129, Torino, Italy

ARTICLE INFO

Keywords:
Nanoscale zero valent iron
Iron reducing bacteria
Trichloroethene
Dehalogenation rate
Cytotoxicity

ABSTRACT

In this work we investigated the interactions between the nanoscale zero valent iron (nZVI) and iron reducing bacteria (IRB) during the remediation of trichloroethene (TCE). Dehalogenation of TCE was examined using nZVI NANOFER 25 (N25) and the Gram-negative bacterium *Shewanella algae* CCM 4595 in liquid and soil slurry microcosms. The rates of dehalogenation were determined by gas chromatography. The interactions between N25 and *S. algae* were investigated using X-Ray diffraction (XRD), ferrozine test, Tandem Scanning Confocal Microscope (TSCM) and viable cell counts. Results showed that *S. algae* impaired TCE removal by N25. TCE dehalogenation rates in liquid microcosms were 17 times faster in absence than in presence of *S. algae*, and 8 times faster in soil slurry microcosms. The concentrations of dissolved Fe^{2+} and Fe^{3+} ions in the N25 + *S. algae* microcosms were an order of magnitude higher than that in bacteria-free microcosms. Moreover, the XRD data showed that the presence of *S. algae* significantly increases the depletion of reactive Fe^0 and causes the formation of poorly soluble and insoluble Fe^{3+} oxide-hydroxides that can inhibit electron transfer from iron to the contaminant. Viable cell counts showed that N25 considerably inhibited microbial growth. Furthermore, TSCM demonstrated that *S. algae* tends to adhere to the surface of N25 suggesting that direct nanoparticle attachment may impact both the microbial population and the electron transfer from the iron to the contaminant. Data suggested that *S. algae* contributes significantly to N25 anoxic corrosion through microbial iron respiration. Our findings provide better insights about the fate of nZVI in the subsurface and its interactions with surrounding microorganisms.

1. Introduction

Tetrachloroethylene (TCE) belongs to the chlorinated solvents which count among the most common groundwater pollutants. The well-known predominance and persistence of these compounds in natural environments have driven extensive research to characterize the reductive dechlorination of chlorinated solvents under various conditions (McCarty, 2010). Attention has been focused on the *in-situ* chemical reduction process using nanoscale metallic iron (Fe^0), also referred to nZVI (Tratnyek and Johnson, 2006). The iron nanoparticles are typically composed of a crystalline α-Fe^0 core and an iron oxide/hydroxide shell (Bruce et al., 2004; Honetschlagerova et al., 2015). A potential limitation of nZVI is its decrease in reactivity due to Fe^0 corrosion and its access within the nanoparticles (Su et al., 2012). Corrosion of Fe^0 in anoxic conditions results in the discharge of Fe^{2+} into the aqueous phase, mass loss of the Fe^0, and formation of corrosion products (Kielemoes et al., 2000). Precipitation of corrosion products on Fe^0 core decreases the porosity of the shell, which limits Fe^0–contaminant interactions (Noubactep, 2008).

Iron reducing bacteria (IRB) (*Geobacter spp.*, *Shewanella spp.*) use insoluble Fe^{3+} oxyhy-droxides and oxides as terminal electron acceptors under anaerobic conditions (Herrera and Videla, 2009). Several studies have shown that *Shewanella algae*, *Shewanella putrefaciens* and *Shewanella oneidensis* promote anoxic corrosion through bacterial respiration (Caccavo et al., 1997; Dubiel et al., 2002) of insoluble ferric ion compounds that protects nZVI from a rapid corrosion. Moreover, the formation of minerals that inhibit direct electron transfer from the iron to the contaminant has been reported for several species of both *Shewanella* and *Geobacter* (Luef et al., 2013). The direct electron transfer can be inhibited also through colonilization of nZVI surface by microorganisms. Alternatively, nZVI can influence microorganisms found in the subsurface. Cathodic H_2 that is produced during the anoxic corrosion process may biostimulate bacterial growth (Liu and Lowry, 2006). Meanwhile, nZVI exerts cytotoxicity via oxidative stress to microorganisms (Auffan et al., 2008; Xiu et al., 2010).

Conflicting conclusions have been reported concerning the effect of

* Corresponding author.
E-mail address: lenka.hokrova@vscht.cz (L. Honetschlägerová).

https://doi.org/10.1016/j.ibiod.2017.10.009
Received 12 September 2017; Received in revised form 26 October 2017; Accepted 26 October 2017
Available online 23 November 2017

IRB on iron corrosion and on the effect of nZVI on bacterial viability. Previous studies with IRB (Shin et al., 2007; Van Nooten et al., 2008; Wang et al., 2014) primarily focused on interactions with microscale ZVI while the interactions between nZVI and IRB have not been studied deeply. One study by Park et al. (2010). examined IRB metabolism in microcosms containing nZVI. Their work suggested that nZVI did not exert serious impact on the microbial population. However, this research did not take into account the presence of a contaminant.

The objective of this work was to investigate the interactions between nZVI and IRB during TCE removal in artificial groundwater (AGW) and in a soil slurry. In the present study *S. algae* CCM 4595 and nZVI NANOFER 25 (N25) were chosen as a model organism and model nZVI, respectively. The experimental microcosms were setup to investigate whether (i) *S. algae* promotes or hinders the removal of TCE using N25; (ii) *S. algae* influences iron corrosion and the precipitation and fractionation of corrosion products; (iii) N25 impairs microbial growth.

2. Materials and methods

2.1. Experimental materials

The chemicals used in this study included Tryptone Soya (TRS) broth (Oxoid, United Kingdom), N_2 (Siad, Czech Republic), $NaHCO_3$ (Penta, Czech Republic), $KHCO_3$ (Lachema, Czech Republic), $MgCl_2.6H_2O$ (Penta, Czech Republic), $CaCl_2.2H_2O$ (Lachema, Czech Republic), methanol (Sigma-Aldrich, Germany), 2,3,5-triphenyltetrazolium (TTC) (Sigma-Aldrich, Germany), ammonium acetate (Penta, Czech Republic), HCl (35%, Penta, Czech Republic), $[NH_3OH]Cl$ (Penta, Czech Republic), TCE (Sigma-Aldrich, Germany). Freshly distilled water was used in all experiments.

The iron nanoparticles used for the experiments (N25) were produced by NANOIRON, Ltd., Czech Republic. *S. algae* CCM 4595 was obtained from the Czech Collection of Microorganisms. The Gram-negative bacterium *S. algae* was chosen as model IRB based on the experiments described in supporting information (SI) (Fig. SI1-SI4). The bacterial strain was grown anaerobically in TRS medium containing lactic acid as an electron donor and Fe(III) citrate as electron acceptor at 30 °C (Gerlach et al., 2000; Venkateswaran et al., 1999). *S. algae* cells were washed twice in distilled water and then re-suspended in AGW. AGW was prepared using demineralized water enriched with 0.5 mM $NaHCO_3$, $KHCO_3$, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$. AGW was sterilized at 121 °C for 15 min and then de-aerated by bubbling with pure nitrogen. For the soil slurry experiments, a natural soil with fraction 0.5–1 mm was used. The soil properties are provided in the supporting information SI5. The soil was sterilized at 121 °C for 30 min twice. All materials such as vials, PTFE septa, syringes, and pipette tips were sterilized at 121 °C for 15 min.

2.2. Experimental setup

Batch experimental microcosms for TCE degradation by N25 and/or *S. algae* were conducted in 20 mL glass vials under anaerobic conditions. The vials were capped with PTFE septa. TCE dehalogenation was examined in both aqueous and soil slurry microcosms. Triplicates were used for all treatments. Each microcosm contained 5 mL of headspace and 15 mL of experimental medium containing AGW or 10 mL AGW + 6.95 g of soil, and either N25 (particle concentration = 3.5 $g.L^{-1}$) and/or *S. algae* (10^5 CFU mL^{-1}) and TCE (10 $mg.L^{-1}$). Control experiments with only 10 $mg.L^{-1}$ TCE in AGW or AGW + SOIL were included. To establish conditions similar to those of natural groundwater the microcosms were incubated on a horizontal shaker, in darkness, at 13 °C for 49 days. The experiments consisted of eight microcosms. The microcosms were prepared separately for each sampling day and they were set up in triplicate. For results average and standard deviation (SD) are reported. The experimental setup was designed based on our previous works (HonetschlÄgerová et al., 2016; Janouškovcová et al., 2012).

TCE and its degradation by-products (dichloroethene (DCE), vinylchloride (VC)) were analyzed using a DANI Master VH gas chromatograph (GC) equipped with a flame ionization detector, a ZB-5 capillary column (Phenomenex Inc.), and a Master SHS static headspace auto-sampler. Peaks were identified by comparison with retention times and quantified relative to response factors of standard compounds. Samples and standard solutions (15 mL) were in 20 mL crimp-cap vials that were incubated at 80 °C for 10 min and head-space samples of 1000 µL were then injected on the column.

Dehalogenation rates of TCE were calculated according to the pseudo-first-order kinetics (Johnson et al., 1996). The removal rate constants (k_{obs}) were determined by fitting the experimental data with the equation of pseudo-first-order kinetics using the method of least square. The k_{obs} values were calculated as the average of triplicate concentration trends, and SD were determined. Statistical analysis was conducted with the OrigiPro 8.5 software. All data were tested for normality (Kolmogorov-Smirnov test). Statistically significant differences between treatment groups were determined by ANOVA with a posteriori Tukey's test. Differences were considered statistically significant when $P \leq 0.05$.

2.3. Phase changes of N25

The effect of *S. algae* on iron corrosion was examined using the ferrozine test. Vials were placed on a magnet and 0.9 mL of supernatant were sampled using a sterile insulin syringe then injected into a cuvette. For Fe^{2+} measurement, 0.1 mL of ferrozine solution and 0.06 mL of buffer solution (10 M ammonium acetate) were added to the iron solution. Absorbance was measured at 562 nm after 5 min using a Jasco V-530 UV-VIS spectrophotometer (Jasco, Japan). For $Fe^{2+} + Fe^{3+}$ measurement, 0.18 mL of reducing agent (1.4 M hydroxylamine in 2 M HCl) and 0.06 mL of buffer solution were added to the mixture from the measurement of Fe^{2+} and the absorbance was measured after 10 min. The precipitation and fractionation of corrosion products were investigated using X-Ray diffraction (XRD). The XRD data were collected with a Bruker AXS D8 θ-θ powder diffractometer with parafocusing Bragg-Brentano geometry using CoKα radiation (λ = 1.79021 Å, U = 34 kV, I = 20 mA). Materials were scanned with a LynxEye ultrafast detector over an angular range 30–80°(2θ) with a step size of 0.0196°(2θ) and a counting time of 19.2 s $step^{-1}$.

2.4. Microbial density and enumeration of live and dead cells

The Most Probable Number (MPN) method was used to determine the viable counts of *S. algae*. MPNs were performed by serial dilutions into 2 mL of sterile TRS medium to which 0.001 mL of coloring agent TTC was added. The number of positive and negative tubes was counted after 48 h of incubation at 22 °C. Log MPN was calculated according to Holliger et al. (1998).

Tandem Scanning Confocal Microscope (TSCM) (Olympus/Andor) was used to examine the changes in cell morphology and the interactions between nZVI and IRB. A rapid fluorescence staining method using LIVE/DEAD® BacLightTM Viability kit (Molecular Probes Inc., Eugene, Oregon, USA) was applied to estimate both viable and non-viable bacteria according to the manufacturer protocol. On the third day of incubation, 1 mL of suspension was taken from the microcosms under sterile conditions and placed in a 2.5 mL Eppendorf tube. Baclight LIVE/DEAD stain (3 µL) was added to the suspension. The cells were enumerated after 15 min of incubation in the dark. Oil immersion objective 100×, 488 and 561 excitation filters and emission filter (Olympus) were used.