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# Impact of the morphology and reactivity of nanoscale zero-valent iron (NZVI) on dechlorinating bacteria

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## ABSTRACT

Nanoscale zero-valent iron (NZVI) is increasingly used for reducing chlorinated organic contaminants in soil or groundwater. However, little is known about what impact the particles will have on the biochemical processes and the indigenous microbial communities. Nanoiron reactivity is affected by the structure and morphology of nanoparticles that complicates the applicability in bioremediation. In this study, the effect of precursors (ferrous sulfate and ferric chloride) and reducing agents (sodium dithionite and sodium borohydride) on the morphology and the reactivity of NZVIs was investigated. We also studied the impact of differently synthesized NZVIs on microbial community, which take part in reductive dechlorination. We demonstrated that both the applied iron precursor and the reducing agent had influence on the structure of the nanoparticles. Spherical nanoparticles with higher $Fe^0$ content (>90%) was observed by using sodium borohydride as reducing agent, while application of sodium dithionite as reducing agent resulted nanostructures with lower $Fe^0$ content (between 68,7 and 85,5%). To determine the influence of differently synthesized NZVIs on cell viability anaerobic enriched microcosm were used. NVZI was used in 0.1 g/L concentration in all batch experiments. Relative amount of *Dehalococcoides*, sulfate reducers (SRBs) and methanogens were measured by quantitative PCR. We found that the relative amount of *Dehalococcoides* slowly decreased in all experiments independently from the precursor and reducing agent, whereas the total amount of microbes increased. The only clear distinction was in relative amount of sulfate reducers which were higher in the presence of NZVIs synthesized from sodium dithionite.

## 1. Introduction

Nanoscale zero-valent iron (NZVI) is one of the most extensively used nanomaterials for the remediation of contaminated soil and groundwater that mainly targets chlorinated organic contaminants (e.g., PCB, TCE, PCE, pesticides, solvents) (Grieger et al., 2010), inorganic anions (e.g., perchlorate) (Cao et al., 2005) or heavy metals (e.g., Cr (VI), Pb(II), As(III), As (V)) (Grieger et al., 2010; Ponder et al., 2000). NZVI is reported as an ideal candidate for in situ remediation because of its large active surface area and high heavy metal adsorption capacity (Tratnyek and Johnson, 2006).

In the past 15 years, various types of NZVI (uncapped, surface-modified and bimetallic NZVI) have been used to develop reductive dechlorination of chlorinated solvents, for dense nonaqueous phase liquid (DNAPL) source zones and to control odor in biosolid treatments (Bezbaruah et al., 2009; Fu et al., 2014; Gong et al., 2015; Xie et al., 2014). However, NZVI is a strong and effective reductant ($E_h = -440$ mV) (Zhang, 2003), recent studies suggest that in situ it may have a biostimulatory or an inhibitory effect (Lee et al., 2008; Li et al., 2010; Xiu et al., 2010b). Although, in the literature some nanoparticles (e.g., Ag, Au, Cu, etc.) (Diao and Yao, 2009; Schrand et al., 2010; Shankar and Rhim, 2014) were reported to affect biological systems at cellular, sub-cellular and protein levels (Farré et al., 2008; Klaine et al., 2008), the mechanism of NZVI toxicity

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towards bacteria is unknown. Different mechanisms have been suggested such as interference with respiration (Lyon et al., 2008), damage of DNA or enzyme inactivation caused by released metal ions (Gogoi et al., 2006), generation of reactive oxygen species (ROS) (Marsalek et al., 2012) and the aforementioned disruption of the cell membrane integrity (Fang et al., 2007). It is known that NZVI inhibits bacterial growth of Escherichia coli by adsorbing and disrupting the cell membranes (Auffan et al., 2008; Lee et al., 2008; Li et al., 2010). The impact of NZVI has investigated mainly on pure cultures (Lee et al., 2008; Xiu et al., 2010a). Limited data is available about the effect of the nanomaterial on indigenous microbial populations of more complex natural systems such as soil microbial population (Fajardo et al., 2012; Kirschling et al., 2010) despite microbial communities take part in reduction of various pollutants (Cao et al., 2005; Grieger et al., 2010; Kirschling et al., 2010; Ponder et al., 2000).

As *Dehalobacter* and *Dehalococcoides* are capable partially or completely dechlorinate PCE or TCE (Duhamel et al., 2004; Holliger et al., 1993) hence the viability of these bacteria is still more important to the full-scale application of site remediation. Several publications reported that NZVI in anaerobic processes could enhance microbial dehalogenation because the evolving $H_2$ during iron corrosion could promote the reduction of halogenated organic compounds (Yin et al., 2012). However, NZVI can be toxic to indigenous bacteria and hinder their participation in the remediation process as inhibitory effect of biological dechlorination in the presence of 1 g/L iron nanoparticles has recently been reported (Diao and Yao, 2009; Xiu et al., 2010b). Xiu et al., 2010 found that bacteria dechlorinating trichloroethylene were initially inhibited by NZVI, but their dechlorination activity and ethane production recovered after a lag period (Xiu et al., 2010b). There is still a debate about the effect of NZVI on methanogens and sulfate reducing bacteria (SRB) under anaerobic conditions (Kirschling et al., 2010). Methanogens, which are known to compete for $H_2$ with dechlorinators (Smatlak et al., 1996; Yang and McCarty, 1998), were significantly stimulated and methane production was increased by adding NZVI, while dechlorinating organisms were inhibited by NZVI (Xiu et al., 2010b). Although NZVI stimulated SRB and methanogen growth in TCE contaminated aquifer materials, in another study, where NZVI was used at the same concentration inhibited pure culture SRB (Shu et al., 2011).

Presently, only a few studies have been reported on the effect of microbial changes on complex ecosystems upon NZVI treatment (Cullen et al., 2011; Fajardo et al., 2012; Kirschling et al., 2010; Němeček et al., 2014; Tilston et al., 2013; Yang et al., 2013). To the best our knowledge, no studies investigated the effect of different NZVIs synthesized using various precursors and reducing agents on microbial communities during the remediation of chlorinated solvents. Therefore, it is important to understand how differently synthesized NZVIs will affect biochemical processes and microbial diversity. In this work, we investigated how the precursors (ferrous sulfate and ferric chloride) and reducing agents (sodium dithionite and sodium borohydride) affect the morphology and reactivity of NZVIs. Here we also demonstrate the impact of differently synthesized NZVIs on the microbial community of a chlorinated hydrocarbon degrading microcosm. We determined if there was any decrease in the microbial abundance and diversity which would indicate NZVI toxicity. We also investigated how NZVIs affect microbial communities with particular regards to the inhibition or biostimulation of microorganisms which play an important role in the remediation process.

## 2. Experimental

### 2.1. Reagents

All chemicals were obtained in high purity and were used as received, including iron(II) sulfate heptahydrate (≥99.0%, Sigma-Aldrich, St. Louis, MO, USA), iron(III) chloride hexahydrate (≥98.0%, Sigma-Aldrich, St. Louis, MO, USA), sodium borohydride (≥99.0%, Sigma-Aldrich, St. Louis, MO, USA), sodium dithionite (≥85.0%, Merck Millipore, Darmstadt, Germany).

### 2.2. NZVIs synthesis

All experiments were carried out under high nitrogen atmosphere for the sake of oxygen exclusion. All aqueous solutions were prepared using deoxygenated and deionized water that was produced by purging high purity nitrogen gas (99.99%) into deionized water for 1 h. Zero-valent iron nanoparticles (NZVIs) were synthesized by reduction of two different iron salts, iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), using two different reducing agents, sodium dithionite ($Na_2S_2O_4$) and sodium borohydride ($NaBH_4$) according to the methods described previously (Wang and Zhang, 1997). The freshly prepared nanoirons were washed to obtain samples free of any reducing agents. The NZVI samples were washed three times with deoxygenated distilled water and were recovered by vacuum-filtration, and washed with ethanol. The efficiency of washing steps (the leftover of the reducing agents) was monitored by HPLC-IC measurement (see S1, Fig. S1 and Table S1). The synthesized NZVI samples were dried and stored in an anaerobic chamber.

The NZVIs formed using ferrous sulfate and ferric chloride as precursors and sodium dithionite as reducing agents were designated as $NZVI_S^{SD}$ and $NZVI_C^{SD}$, respectively, and NZVIs formed by sodium borohydride as reducing agent were designated as $NZVI_S^{SB}$ and $NZVI_C^{SD}$, respectively.

### 2.3. NZVI particles reactivity

The $Fe^0$ content in particles was determined by measuring the $H_2$ gas evolved during particle digestion in a concentrated $H_2SO_4$ solution. The volume of $H_2$ was quantitatively measured using a gas burette with pressure equalizing against atmospheric pressure. The reported $Fe^0$ content of the NZVI samples was obtained from three replicate measurements.

### 2.4. Morphological analysis

In order to investigate morphology of the synthesized NZVIs, transmission electron microscopy (TEM) was used. The TEM observations were performed on a FEI Tecnai G2 20 X-Twin instrument (200 kV accelerating voltage) using copper mounted holey carbon grids.

The size distribution of NZVI particles was determined by image analysis of TEM pictures using ImageJ software (http://rsbweb.nih.gov/). Five representative images of equal magnification, taken at different spots of the TEM grid, were first subjected to rolling ball background subtraction and contrast enhancement, and then the diameter of the metal nanoparticles in the image was manually measured against the calibrated TEM scale bar. Each diameter distribution was constructed from 50 individual nanoparticle or nanowire diameters.

### 2.5. Crystal structure

X-ray diffraction (XRD) was used to investigate the crystalline