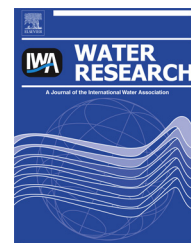


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Inhibition of sulfate reducing bacteria in aquifer sediment by iron nanoparticles

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ABSTRACT

Batch microcosms were setup to determine the impact of different sized zero valent iron (Fe⁰) particles on microbial sulfate reduction during the *in situ* bio-precipitation of metals. The microcosms were constructed with aquifer sediment and groundwater from a low pH (3.1), heavy-metal contaminated aquifer. Nano (nFe⁰), micro (mFe⁰) and granular (gFe⁰) sized Fe⁰ particles were added to separate microcosms. Additionally, selected microcosms were also amended with glycerol as a C-source for sulfate-reducing bacteria. In addition to metal removal, Fe⁰ in microcosms also raised the pH from 3.1 to 6.5, and decreased the oxidation redox potential from initial values of 249 to –226 mV, providing more favorable conditions for microbial sulfate reduction. mFe⁰ and gFe⁰ in combination with glycerol were found to enhance microbial sulfate reduction. However, no sulfate reduction occurred in the controls without Fe⁰ or in the microcosm amended with nFe⁰. A separate dose test confirmed the inhibition for sulfate reduction in presence of nFe⁰. Hydrogen produced by Fe⁰ was not capable of supporting microbial sulfate reduction as a lone electron donor in this study. Microbial analysis revealed that the addition of Fe⁰ and glycerol shifted the microbial community towards *Desulfosporosinus* sp. from a population initially dominated by low pH and metal-resisting *Acidithiobacillus ferrooxidans*.

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1. Introduction

Heavy metals are natural component of the earth's crust. Although some are essential micronutrients for life, at increased concentrations they also lead to severe poisoning. Compared to organic pollutants, heavy metals never degrade in the environment, but are only transferred and transformed

(Satyawali et al., 2010; Hashim et al., 2011). Over time, heavy metals can become mobile due to changes in their speciation and/or soil pH, and as a result can leach into aquifers causing groundwater contamination (Alloway, 1990). Over the past few decades, numerous technologies have been developed to deal with heavy-metal contaminated groundwater, such as chemical precipitation, electrocoagulation, and *in situ* bio

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precipitation (ISBP) using sulfate reduction (Diels et al., 2002; Vanbroekhoven et al., 2008). ISBP immobilizes heavy metals in groundwater as relatively stable precipitates (mainly sulfides). The process works by inducing sulfate-reducing bacteria (SRB), which produce sulfide and ultimately poorly soluble metal-sulfides (Diels et al., 2002). SRBs are considered very efficient for heavy metal remediation (Jong and Parry, 2003), however field trials are often not successful (Bécharde et al., 1994). One reason for the lack of success in field trials is that microbial processes generally require optimal environmental parameters (pH, redox potential, sulfate, temperature, suitable electron donor) for sustainable growth, which are often not met in natural conditions. In that case, additional reactive materials need to be provided to maintain a suitable growth environment for the bacteria. In general, rich carbon sources, such as lactate, acetate, glycerol, molasses, manure, wine waste etc. are injected in the aquifer (Martins et al., 2009; Satyawali et al., 2010; Castillo et al., 2012). In some sulfate poor systems, sulfate can also be added to stimulate SRBs.

Heavy metal removal using Fe⁰ for dissolved water contaminant remediation has been demonstrated by both laboratory and field tests (Wilkin and McNeil, 2003; Dries et al., 2005a; Burghardt and Kassahun, 2005). The preliminary idea of using metallic iron for the contaminant removal of redox active contaminants is based on the contaminant reduction through electron transfer during Fe⁰ oxidation (Weber, 1996). Toxic metal removal by Fe⁰ takes place through a combination of surface adsorption, precipitation and co-precipitation with iron oxides (Gu et al., 1998; Huang et al., 2013). Colloidal to granular sizes of Fe⁰ are typically used, depending on the technology employed for remediation. For example, granular Fe⁰ is often used to create permeable barriers across the flow path of a contaminated groundwater plume. On the other hand, nano and micro scale Fe⁰ can be directly injected into natural aquifers, which is often considered as an advantage over barrier technology since no excavation of contaminated soil is required. Interest has grown towards the feasibility of using an integrated Fe⁰ and ISBP mechanism to improve groundwater remediation and stability of removed precipitates (Gandhi et al., 2002; Gu et al., 2002; Fernandez-Sanchez et al., 2004; Xin et al., 2008). In addition to direct contaminant reduction at the surface, Fe⁰ particles may also be able to stimulate SRB by depleting O₂, lowering the redox potential, and producing water derived H₂ via corrosion reactions which can also be used as an electron donor by SRB (Karri et al., 2005). Formation of stable metal-sulfide precipitates through sulfate reduction has been well documented (Geets et al., 2006; Cruz Viggì et al., 2010; Battaglia-Brunet et al., 2012). So an integrated Fe⁰-SRB process could be of greater interest in sustainable groundwater remediation.

The particle size and dosing of Fe⁰ are highly important for optimizing the removal process. These two parameters determine the available reactive surface area, pH changes, and H₂ availability. There are only few studies that examine the impact of Fe⁰ on bio-geochemical dynamics in natural aquifer systems (Wei et al., 2010; Kirschling et al., 2010; Tilston et al., 2013), but there is no direct study related to the impact of different size of Fe⁰ on subsurface microbiology, particularly on SRBs. Therefore, there is a lack of information on how Fe⁰

impacts microbial processes in such environments. In this study, we performed a series of batch experiments using three different particle sizes of Fe⁰, with the primary aim of understanding the interaction between Fe⁰ and sulfate-reducing bacteria during ISBP for groundwater contaminant removal.

2. Material and methods

2.1. Groundwater and aquifer sediment

The groundwater (GW) and aquifer sediment were collected from a heavy-metal contaminated site in Belgium at a depth of ≈32 m. The chemical and physical properties of sediment are provided in Table 1. Further details of this site have been published elsewhere (Vanbroekhoven et al., 2008). Groundwater and sediment were preserved in airtight glass containers, and stored in the dark at 4 °C until use.

2.2. Zero valent iron particle

Three different sizes of Fe⁰ were used for this study: granular Fe⁰ (gFe⁰, Götthard Maier, Germany), micro Fe⁰ (mFe⁰, Högenäs, Sweden) and nano Fe⁰ (nFe⁰, Toda kogyo Corp, Japan) with an average particle size of 0.25–2 mm, 20–40 μm and 70–100 nm, respectively (information provided by manufacturer). Particles were used as received without pre-treatment.

2.3. Batch experiment setup

Metal bio-precipitation experiments were performed in 250 mL serum bottles, comprising 40 g of aquifer sediment suspended in 200 mL of groundwater. For each type of Fe⁰, 4 experimental conditions were setup: (i) aquifer sediment + GW + Fe⁰, (ii) aquifer sediment + GW + Fe⁰ + glycerol, (iii)

Table 1 – Groundwater and Aquifer sediment characteristics.

Groundwater	
pH	3.1
ORP	326 mV
EC	987 μS cm ⁻¹
Dissolved oxygen	0.13 mg L ⁻¹
Dissolved sulfate	420 mg L ⁻¹
Zinc	49 mg L ⁻¹
Cadmium	0.42 mg L ⁻¹
Fe	7.4 mg L ⁻¹
As	0.04 mg L ⁻¹
Chloride	21 mg L ⁻¹
Nitrate (as Nitrogen)	1 mg L ⁻¹
Total hardness	2.4 mmol L ⁻¹
Total organic carbon	2.7 mg L ⁻¹
Aquifer sediment	
pH	4.18
Total organic carbon	0.022
Total S	219 (mg S kg ⁻¹)
Mn	16 (mg kg ⁻¹)
Fe	650 (mg kg ⁻¹)
Cd	0.6 (mg kg ⁻¹)
Zn	41 (mg kg ⁻¹)
As	75 (mg kg ⁻¹)

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