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Use of CAH-degrading bacteria as test-organisms for evaluating the impact of fine zerovalent iron particles on the anaerobic subsurface environment

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HIGHLIGHTS

- ATP-measurements have potential for standardized anaerobic ecotoxicity test.
- CAH-degrading mixed bacteria were used as relevant test-organisms.
- nZVI doses >0.05 g L^{-1} show inhibiting effects on bacterial community.
- mZVI doses >15 g L^{-1} show inhibiting effects on bacterial community.
- Bacterial community inhibition corresponds to a pH increase above 7.5.

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ABSTRACT

The release of fine zerovalent iron (ZVI) particles in the environment after being introduced for in-situ treatment of compounds like chlorinated aliphatic hydrocarbons (CAHs) may raise questions toward environmental safety, especially for nanoscale materials. Classical single-species ecotoxicity tests do focus on aerobic conditions and are only relevant for the scenario when ZVI-particles reach surface water. Herein, we present an alternative approach where a CAH-degrading mixed bacterial culture was used as test-organisms relevant for the anaerobic subsurface. The impact of different ZVI particles on the bacterial culture was evaluated mainly by quantifying ATP, a reporter molecule giving a general indication of the microbial activity. These lab-scale batch tests were performed in liquid medium, without protecting and buffering aquifer material, as such representing worst-case scenario. The activity of the bacterial culture was negatively influenced by nanoscale zerovalent iron at doses as low as 0.05 g L^{-1}. On the other hand, concentrations up to 2 g L^{-1} of several different types of microscale zerovalent iron (mZVI) particles stimulated the activity. However, very high doses of 15–30 g L^{-1} of mZVI showed an inhibiting effect on the bacterial community. Negative effects of ZVIs were confirmed by H_2 accumulation in the batch reactors and the absence of lactate consumption. Observed inhibition also corresponded to a pH increase above 7.5, explicable by ZVI corrosion that was found to be dose-dependent. The obtained results suggest that low doses of mZVIs will not show severe inhibition effects on the microbial community once used for in-situ treatment of CAHs.

1. Introduction

The more frequent use of microscale and nanoscale zerovalent iron particles (mZVI and nZVI, respectively) for in-situ remediation (Gillham and O'Hannesin, 1994; Matheson and Tratnyek, 1994; Zolla et al., 2009) of chlorinated aliphatic hydrocarbons (CAHs) may raise concerns related to environmental, health and safety aspects (Auffan et al., 2009), as significant amounts of mZVIs and nZVIs are directly injected into the saturated subsurface (Zhang, 2003; Truex et al., 2011).

Although the reactivity of mZVIs and nZVIs in respect to their CAH-removal efficiency has been extensively reported (Velimirovic et al., 2013), studies reporting the impact of mZVIs and nZVIs on the anaerobic subsurface environment are scarce. Selection of representative approaches to evaluate potential ecotoxicity effects of zerovalent iron (ZVI) appears to be challenging

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Table 1
Characteristics of studied ZVI particles.

Iron name	PSD [D_{10}, D_{50}, D_{90}][a] (µm)	BET[b] ($m^2\ g^{-1}$)	Carbon content (wt%)[c]	Oxygen content (wt%)	Sulfur content (wt%)	Nitrogen content (wt%)	Supplied by	Form
mZVI-1	22, 41, 62	0.09	0.00	0.09	0.01	0.00	Höganäs (SE)	Powder
mZVI-2	1, 5, 12	1.10	0.01	0.71	0.00	0.00		
mZVI-3	2, 30, 59	0.51	0.28	0.58	0.00	0.00		
mZVI-4	24, 56, 99	0.06	0.02	1.10	0.01	0.01		
mZVI-5	1, 3, 9	nm	0.01	1.79	0.01	0.00		
HQ [d]	0.6, 1.2, 2.4	0.82	0.75	0.44	0.00	0.75	BASF (DE)	Powder
NF25s [d]	D_{50} < 0.05[e]	25.0[e]	0–1[e]	nm	nm	nm	NANOIRON (CZ)	Suspension

nm – not measured.
[a] Particle Size Distribution measured by laser diffraction with a Sympatec Helos/Rodos dry particle size analyzer.
[b] BET: Specific Surface Area analyzed with a Micromeritics Flowsorb II according to the Brunauer–Emmett–Teller (single point measurement).
[c] The bulk concentration of C, O, N and S in the iron powder. Elemental analyses of carbon and sulfur were analyzed by combustion analysis with a CS-400 LECO. An elemental analysis of oxygen and nitrogen was analyzed by an inert gas fusion principle with a TC-436 LECO. The accuracy of analyses was ± 0.01%.
[d] Commercially available.
[e] Producers data.

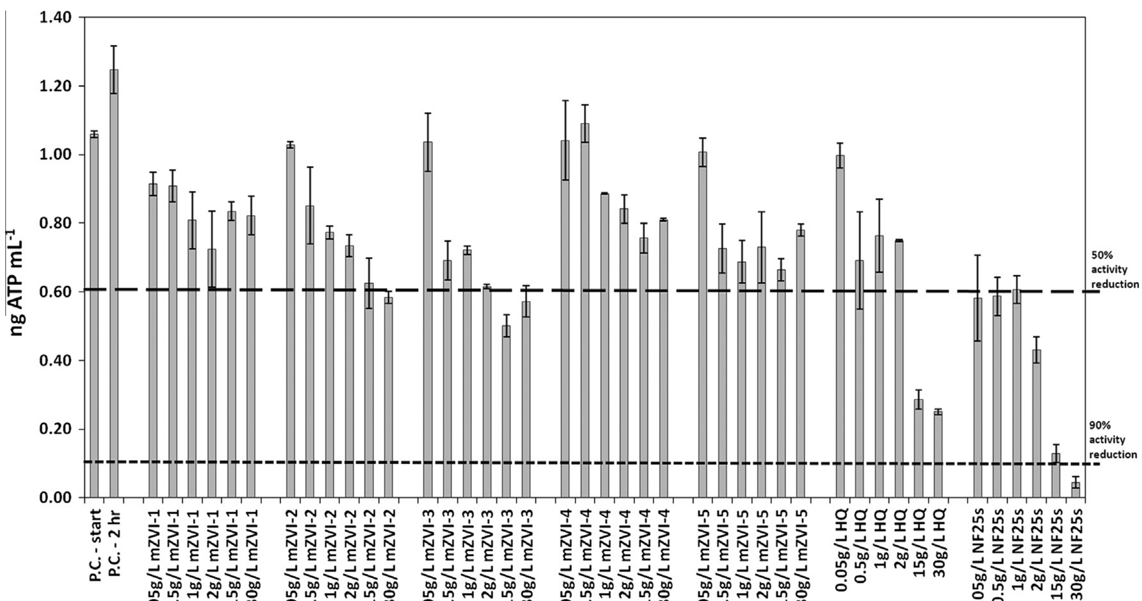

Fig. 1. Short-term changes in general microbial activity (ng ATP mL^{-1}) when exposed 2 h to a range of different concentrations of ZVIs. Data points represent the mean of two replicates with the standard error of the mean. P.C. represents positive control.

as classical single-species toxicity tests (OECD or ISO standard tests) and molecular approaches focus mainly on aerobic aquatic environment (e.g. *Cyanobacteria*) and soil biota (e.g. *Escherichia coli*, earthworms, nematodes) (Auffan et al., 2008; Lee et al., 2008; Diao and Yao, 2009; Li et al., 2010; Chen et al., 2011; Cullen et al., 2011; Marsalek et al., 2012; Fajardo et al., 2013; Saccà et al., 2014). On the other hand, limited research has been conducted on potential impacts of fine ZVIs on anaerobic microorganisms relevant for the anaerobic subsurface environment and CAHs biodegradation processes (e.g. methanogens, sulfate reducing bacteria and CAH-degrading bacteria) (Barnes et al., 2010; Kirschling et al., 2010; Xiu et al., 2010; Kumar et al., 2014). As a consequence of no standardized ecotoxicity tests, impact of fine ZVIs on the saturated anaerobic subsurface was evaluated by following inhibition on contaminant removal and using molecular techniques (e.g. plate counting, DGGE and qPCR) (Kirschling et al., 2010).

Therefore, the objective of this study was to propose a new approach for evaluation of potential impacts of fine ZVIs on the anaerobic subsurface compartment and dechlorination activity of indigenous microorganisms by using (1) an anaerobic CAH-degrading mixed bacterial culture as test-organisms and (2) commercially available adenosine tri-phosphate (ATP) assay used for fast and rapid screening of the general metabolic activity of microorganisms (Hammes et al., 2010). More specifically, to understand the effect of fine ZVIs on the activity of the test-organisms during in-situ remediation, a dose–response lab-scale batch test procedure was elaborated. As no potentially protecting aquifer material is present, the test represents a worst-case scenario. Besides ATP assay, the effect of different ZVIs on the target-organisms was also evaluated by monitoring biologically mediated degradation of trichloroethylene (TCE) as specific activity indicator using different chemo-analytical methods.

2. Materials and methods

2.1. Bacterial culture

The CAH-degrading mixed bacterial culture was enriched from trichloroethene (TCE) contaminated aquifer (site A, Belgium) via transfers in an anaerobic mineral medium (Haest et al., 2011)