Journal of Hazardous Materials 308 (2016) 106–112

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Particles and enzymes: Combining nanoscale zero valent iron and organochlorine respiring bacteria for the detoxification of chloroethane mixtures

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HIGHLIGHTS

- A combination of nZVI and ORB to treat mixed chloroethanes is examined.
- Mixed vitamins and L-cysteine enhance nZVI reactivity toward 1,1,2-TCA.
- ORB are sensitive to nZVI above 0.1 g/L.
- ORB are completely inhibited by nZVI above 0.5 g/L.
- nZVI below 0.05 g/L stimulates dechlorination of mixed chloroethanes by ORB.

ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form
16 November 2015
Accepted 20 December 2015
Available online 28 December 2015

Keywords:
Nanoscale zero valent iron
Organochlorine respiring bacteria
Bioaugmentation
Chloroethane
Reductive dechlorination

ABSTRACT

Nanoscale zero valent iron (nZVI) and organochlorine respiring bacteria (ORB) are two technologies used to detoxify chlorinated aliphatic hydrocarbons (CAHs). nZVI can rapidly detoxify high CAH concentrations, but is quickly oxidised and unable to degrade certain CAHs (*e.g.,* 1,2-dichlorothane). In contrast, ORB can dechlorinate CAHs resistant to nZVI (*e.g.,* 1,2-dichlorothane) but are inhibited by other CAHs of concern degradable by nZVI (*e.g.,* chloroform and carbon tetrachloride). Combining the two was proposed as a unique treatment train to overcome each technology's shortcomings. In this study, this combined remedy was investigated using a mixture of 1,2-dichloroethane, degradable by ORB but not nZVI, and 1,1,2-trichloroethane, susceptible to both. Results indicated that nZVI rapidly dechlorinated 1,1,2-trichloroethane when supplied above 0.5 g/L, however ORB were inhibited and unable to dechlorinate 1,2-dichloroethane. pH increase and ionic species associated with nZVI did not significantly impact ORB, pinpointing Fe^0 particles as responsible for ORB inhibition. Below 0.05 g/L nZVI, ORB activity was stimulated. Results suggest that combining ORB and nZVI at appropriate doses can potentially treat a wider range of CAHs than each individual remedy. At field sites where nZVI was applied, it is likely that *in situ* nZVI concentrations were below the threshold of negative consequences.

1. Introduction

Chlorinated aliphatic hydrocarbons (CAHs) pose a significant threat to water resources and human health worldwide. Many are toxic to the nervous system, cause liver and kidney disease and are classified by the USEPA as probable carcinogens [1,2]. Of particular focus in this study are 1,2-dichloroethane (DCA) and 1,1,2-trichloroethane (TCA), with respective Maximum Contaminant Level values in drinking water of 0.005 mg/L and 0.006 mg/L. In the United States, DCA and TCA were found at 412 and 169 sites respectively out of 1322 National Priorities List contaminated sites in 2015 [3]. Given the extensive pollution and potential severe health effects caused by these CAHs, there is considerable interest in rapid and effective remediation technologies.

Reductive dechlorination is a process which transforms CAHs to less or non-toxic intermediates and end products and is the basis

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http://dx.doi.org/10.1016/j.jhazmat.2015.12.036
0304-3894/

for several remediation methods. In the last two decades, nanoscale zerovalent iron (nZVI) has been successfully employed to reductively dechlorinate a variety of CAHs [4,5]. nZVI particles possess a significant surface area to weight ratio, which leads to a large density of reactive sites and contaminant removal capacity. They can also be injected as slurry directly in the subsurface for source zone remediation. Particles are often coated with stabilizers such as carboxymethyl cellulose (CMC) to improve stability and subsurface transport [6]. Once injected into the groundwater, nZVI reacts with various contaminants and groundwater constituents and is oxidized to form iron oxides and other iron-bearing minerals. Although nZVI can reduce most CAHs (including TCA), it is unable to dechlorinate DCA [5].

Reductive dechlorination is also carried out by anaerobic organochlorine respiring bacteria (ORB) as part of their metabolism. Reductive dehalogenase enzymes specifically bind certain chlorinated compounds and transfer electrons to them at the end of respiratory chains, thereby releasing chloride ions [7]. In contrast to Fe^0, ORB can dechlorinate DCA: species comprise *Desulfitobacterium dichloroeliminans* strain DCA1 [8], *Dehalococcoides mccartyi* [9], *Dehalogenimonas* spp. [10]. and *Dehalobacter* spp. [11]. TCA is degraded by certain strains of *Dehalobacter* and *Dehalogenimonas* [10,12].

While bioremediation using ORB is now a proven technology [13], certain weak points remain. These include the sequential removal of mixtures, as most ORB can utilize several CAHs but preferentially dechlorinate the more heavily chlorinated ones which yield more energy, resulting in a slower process [14,15]. Other problematic situations are the presence of ORB-inhibiting co-contaminant CAHs (*e.g.,* carbon tetrachloride, chloroform and 1,1,1-TCA) [16,17], ORB absence from contaminated sites, and conditions hostile to anaerobic microbial life (oxygen, low/high pH, lack of nutrients *etc.*).

In these cases, a combination of nZVI injection and ORB stimulation/augmentation presents a potential solution coupling the benefits of the two methods while negating their drawbacks. This would entail nZVI degrading certain CAHs while fostering favorable conditions for ORB by lowering redox potential, increasing pH, detoxifying co-contaminants and providing electron donors in the shape of polymer coatings and hydrogen [18]; and ORB degrading those CAHs resistant to nZVI-driven dechlorination. However, nZVI toxicity to bacteria has been reported [19,20]. and concerns remain over long-term effects of releasing nanoparticles in the environment [21], representing potential challenges to this treatment method.

In this study, the feasibility and effectiveness of such a combined strategy was evaluated in the context of treating a mixture of DCA and TCA. Initially, nZVI and ORB were compared separately in terms of their dechlorinating capability. Upon combining the two, a negative impact of nZVI on ORB activity was observed. This prompted an investigation of the response of ORB to separate effects of the nZVI slurry, namely pH increase and presence of boron, sulphate and iron species. Finally, the combination of nZVI and ORB over a range of nZVI concentrations was examined both in terms of dechlorination performance and cell toxicity.

2. Materials and methods

2.1. Enrichment procedure and community profiling

A sediment-free, methanogen-free enrichment culture was obtained from soil from a chlorinated compound-contaminated facility at Botany Bay, Sydney, Australia. Approximately 2 g of wet soil was placed under N_2–CO_2 (80/20 v/v) in a 160 mL serum bottle with 100 mL of basal anaerobic medium prepared according to Löffler et al. [22], with 5 mM ethanol as electron donor and 5 mM bromoethanesulfonic acid (BES) to inhibit methanogenesis. The culture was amended with 500 μM 1,2-DCA and 500 μM 1,1,2-TCA (Sigma–Aldrich) and incubated statically in the dark at room temperature. Following complete dechlorination of DCA and TCA to ethene, the culture was transferred (5% v/v) into fresh medium. A total of three transfers were carried out before inoculation.

Prior to inoculation, genomic DNA was extracted from 5 mL of culture using the PowerSoil® DNA Isolation Kit (MoBio Inc.) following the manufacturer's instructions. Ribosomal tag pyrosequencing of partial 16S rRNA gene sequences was carried out at the Research and Testing Laboratory (Lubbock, TX, USA). Primers 27f and 519r [23]. were used to PCR amplify a 500-bp product spanning the 16 S rRNA bacterial variable regions V1–V3. Sequencing was performed on a Roche GS FLX Titanium machine. Resulting sequences were checked for quality and sequences shorter than 250 base pairs were discarded using MOTHUR [24], chimeras were checked using the Uchime algorithm [25], and remaining sequences were classified by the Ribosomal Database Project (https://rdp.cme.msu.edu/) naive classifier with 80% confidence trained with Silva reference dataset 6.

2.2. nZVI synthesis

nZVI particles were synthesized using a water-based approach [26]. A food grade anionic polymer (3% w/v of 90,000 M sodium carboxymethyl cellulose, or CMC) was dissolved overnight in deionized water and then deoxygenated with N_2 gas. Freshly prepared $FeSO_4{\cdot}7H_2O$ solution was added to the CMC solution to yield 2.5 g/L Fe^{2+} and 1.2% CMC (w/v). The mixture was stirred for at least 15 min to ensure the formation of the Fe^{2+} – CMC complex. nZVI particles were then synthesized by reducing Fe^{2+} ions (Fe^{2+} – CMC solution) by dropwise addition of $NaBH_4$ solution (BH_4^-/Fe^{2+} molar ratio of 2) with continuous stirring. The suspension was gently agitated for an additional 30 min to ensure complete nucleation of nZVI particles on the polymer. nZVI synthesis was conducted in an anaerobic chamber maintaining an O_2-free environment by purging with O_2-free Ar-H_2 (95/5 v/v).

2.3. Incubations

All experiments were carried out in triplicate 120 mL (nominal capacity) serum bottles (Wheaton) capped with Teflon-lined septa (Wheaton) and aluminium crimps containing 75 mL of water or medium. The medium (Table 1) was prepared according to Löffler et al. [22].

Concentrations were always adjusted so that following dilution with nZVI slurry or other amendments, the final concentration would be as shown in Table 1. Where applicable, the ORB culture was consistently added as a 20 mL amendment and fed with 5 mM ethanol and 1 mM acetate. Treatments were incubated statically in the dark at room temperature.

2.4. Viability assay

The ORB culture was exposed to 0, 0.05, 0.1, 0.2, 0.5 and 1 g/L nZVI in triplicate, consistent with concentrations expected during field application of nZVI [27]. Immediately upon inoculation and every 2–3 days thereafter, viable heterotrophic counts were determined using a modified Miles and Misra method. In brief, three 10 μL drops of undiluted sample and 10^{-1} to 10^{-7} dilutions were pipetted on R2A agar plates (Oxoid). Plates were incubated at room temperature for 48 h before colony-forming-units were counted.