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Sulfur and oxygen isotope tracing in zero valent iron based *In situ* remediation system for metal contaminants

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HIGHLIGHTS

- We followed sulfur and oxygen isotope fractionation of dissolved sulfate molecule in groundwater.
- Sediment was incubated with zero valent iron in flow through columns.
- Microbial sulfate reduction was observed.
- A good relationship between $\delta^{34}S$ and sulfate reduction rate was obtained.
- A linear relationship is between $\delta^{34}S$ and $\delta^{18}O$ was observed.

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ABSTRACT

In the present study, controlled laboratory column experiments were conducted to understand the biogeochemical changes during the microbial sulfate reduction. Sulfur and oxygen isotopes of sulfate were followed during sulfate reduction in zero valent iron incubated flow through columns at a constant temperature of 20 ± 1 °C for 90 d. Sulfur isotope signatures show considerable variation during biological sulfate reduction in our columns in comparison to abiotic columns where no changes were observed. The magnitude of the enrichment in $\delta^{34}S$ values ranged from 9.4‰ to 10.3‰ compared to initial value of 2.3‰, having total fractionation δS between biotic and abiotic columns as much as 6.1‰. Sulfur isotope fractionation was directly proportional to the sulfate reduction rates in the columns. Oxygen isotopes in this experiment seem less sensitive to microbial activities and more likely to be influenced by isotopic exchange with ambient water. A linear relationship is observed between $\delta^{34}S$ and $\delta^{18}O$ in biotic conditions and we also highlight a good relationship between $\delta^{34}S$ and sulfate reduction rate in biotic columns.

1. Introduction

In situ groundwater remediation of metal contaminants has emerged as a sustainable option in recent years for various reasons, e.g. economic feasibility and energy consumption in pump and treats methods, site accessibility, etc. (Farhadian et al., 2008). For *in situ* remediation, various possibilities have also been explored for instance by enhancing natural attenuation, providing electron donors (Satyawali et al., 2010) or by using reactive barrier materials (Benner et al., 1999; Waybrant et al., 2002). Zero valent iron (Fe°) is getting large attention lately as a reactive material for *in situ* applications (Wilkin and McNeil, 2003; Dries et al., 2005; Burghardt and Kassahun, 2005). The highly reducing nature and relatively larger available specific surface area makes Fe° a suitable medium for groundwater contaminant removal. There have been already many successful installation of Fe° based remediation system in last decade (Gu et al., 1998; Rowland, 2002; Liang et al., 2003; Phillips et al., 2010). Fe° has been successfully used in lab scale as well as field scale applications, dealing with wide range of groundwater contaminants e.g. chlorinated compounds and metals (Dries et al., 2005; Doong and Lai, 2006; Habekost and Aristov, 2012), radioactive material (Burghardt and Kassahun, 2005; Klimkova et al., 2011), metalloid, e.g. arsenic (Su and Puls, 2003; Lien and Wilkin, 2005; Biterna et al., 2010), pharmaceuticals and pesticides (Keum and Li, 2004; Bautitz et al., 2012). However, the biogeochemical dynamics and contaminants behavior in

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subsurface environments are still poorly understood, as real field sites often encounter problems due to little or no control over fluxes and changes in subsurface processes with time or seasons. Regular chemical analysis and monitoring may not be a practical or economic option in many isolated sites. These complications often make it difficult to understand the actual processes (biotic/abiotic) contributing in contaminant removal, needless to say that this distinction is rather very important in designing realistic remediation strategy for any particular site.

Stable isotopes have emerged as a potential tool in understanding the dynamics of pollutants in natural systems (Fritz et al., 1989; Slater et al., 2002; Banas et al., 2009). Relatively easy analysis and bulk information makes isotope study a practical and viable option. The characterization and quantification of electron-accepting processes, like nitrate, iron and sulfate reduction are extremely valuable in estimating the sustainability and longevity of degradation processes in any subsurface system (Knöller et al., 2006). This characterization is of primary importance in case of *in situ* treatment process, where there is little control over changing processes in subsurface environments. Biological sulfate reduction has been reported earlier in similar Fe° based treatments systems (Gu et al., 2002; Van Nooten et al., 2007; Xin et al., 2008). Quantification of sulfate reduction by following dissolved concentrations of sulfate and co-existing sulfide in groundwater is often a challenge due to possible dilution, mixing or mineral precipitation processes. Precipitation of dissolved sulfide is particularly important in case of Fe° based systems where abundant Fe(II) is available in groundwater for possible FeS precipitation. It has been shown that sulfate reducing bacteria (SRB) preferentially remove lighter isotopes of sulfur and oxygen from the sulfate molecule resulting into isotopic enrichments of both heavier isotopes i.e. ^{18}O and ^{34}S in residual sulfate (Fritz et al., 1989).

In this study, we followed the evolution of $\delta^{34}S$ and $\delta^{18}O$ from dissolved sulfate in groundwater within lab scale Fe° based flow through columns, which were primarily designed for heavy metal removal from groundwater. The aim of this study was to characterize the isotope fractionation and changes during a long-term treatment processes and also to determine the impact of microbiology on isotopic signature in subsurface redox environment. We summarize here the results of sulfur and oxygen isotope fractionation in Fe° based lab scale *in situ* removal systems over 90 d of experiment.

2. Material and methods

2.1. Column design

Four double jacketed flow through glass columns (30 cm length × 4.5 cm id, total liquid volume = 480 mL) were setup in laboratory for ~30 week at controlled temperature (20 ± 1 °C), with the primary aim of groundwater contaminant removal using Fe° as a reactive material. Columns were filled with sediment obtained from a heavy metal contaminated site in Belgium from a depth of ≈32 m, more description about this site is given elsewhere (Vanbroekhoven et al., 2008). Efforts were made to design a lab scale concept of *in situ* reactive barrier using two types of Fe° differing in particles size and source, i.e. granular zero valent iron (gFe°, Gotthard Maier, Germany) and micro zero valent iron (mFe°, Högenäs, Sweden) with an average particle size of 0.25–2 mm and 20–40 μm, respectively. For each column, the first bottom half (~240 mL) was filled with an aquifer/Fe° mixture with ratios of 80:20 and 98:2 v/v for gFe° and mFe°, respectively. The second (upper) half of all columns was filled only with aquifer (in Supplementary Material (SM), Fig. SM-1). Filling of columns was performed under nitrogen atmosphere in a glove box. Simulated groundwater, which was prepared in lab conditions corresponding to the site characteristics (Table SM-1), was injected in parallel through the columns using a peristaltic pump at a constant flow rate of 1 ± 0.2 mL h^{-1}. A slight over pressure (1 kPa) of N_2 was maintained in the feeding bottles to avoid air contact during column feeding. All tubes and fittings used in the experiment were acid washed and flushed with nitrogen before use.

For each Fe° type, two columns were set-up, of which one was fed with a small dose of glycerol (0.1% v/v of inlet water) to enhance indigenous microbial activity and the other columns was exposed to gamma radiation (Ionisos, Dagneux, France), with minimum absorbed radiation dose of 25 kGy, before injecting groundwater to restrict all microbial activities.

2.2. Analytical methods

Defined volumes of samples (from 50 to 1000 μL) were extracted from columns using a nitrogen filled plastic syringe, by injecting the nitrogen and extracting equal amount of liquid from column prior to observation and counting of bacterial cells. Samples were immediately diluted in deionized water and filtered onto a black polycarbonate filter, 0.22 μm (Nuclepore, Whatman, Kent, UK). The filter was incubated 15 min in the dark with 1 mL filtered deionized water mixed with 1 μL DAPI (4′, 6-diamidino-2-phenylindole) solution (1 mg mL^{-1}, Sigma). This mixture was removed by filtration, and the filter was rinsed two times with 1 mL filtered deionized water. The filter was then mounted on a glass slide with Citifluor (Biovailey, France), and observed with an optical microscope (Zeiss Axio Imager Z1) equipped with Filter Set 49 for DAPI, UV HBO lamp and a digital camera. Bacteria were enumerated on 10 independent fields (each of 5800 μm^2). Cell counts were calculated considering the volume of the sample used and filter surface area calculations on an average basis. Sulfate concentration was analyzed with a spectrophotometer operating at λ 540° using specific analysis kits (Merck Spectroquant kit 1.14548.001, Germany).

Samples for sulfur and oxygen isotope analysis were collected at the outlet of columns using 250 mL pre-acid washed plastic perplex bottles. Cd-Acetate was already added in the bottles (5% v/v) prior to sample collection, to fix sulfur as CdS, and then the aliquot was filtered through a 0.2 μm nitrocellulose filter before chemical determination of residual sulfate. The amount of sample collected varied at different time points during the experiment as the sulfate concentration in the outlet solution changed over the time. However, in any case, a minimum of 5 mg of SO_4 was collected for every sampling point. The analysis was performed as described by (Fritz et al., 1989).

Dissolved sulfate was precipitated as $BaSO_4$ at pH < 4 (in order to remove HCO_3^- and CO_3^{2-} species) by adding a $BaCl_2$ solution. The isotopic analyses on $BaSO_4$ were carried out using a Delta + XP mass spectrometer coupled in continuous-flow mode to a Thermo Elemental Analyzer in BRGM laboratories. Sulfate-isotope compositions are reported in the usual δ-scale in ‰ with reference to V-CDT (Vienna Canyon Diablo Troilite) and V-SMOW (Vienna Standard Mean Ocean Water) according to δsample (‰) = {(*R*sample/*R*standard) − 1}1000, where *R* is the $^{34}S/^{32}S$ and $^{18}O/^{16}O$ the atomic ratios. Sulfate-isotope compositions ($\delta^{34}S$ (SO_4) and $\delta^{18}O$ (SO_4)) were measured with a precision of ±0.3‰ vs. CDT for $\delta^{34}S$ (SO_4) and ±0.8‰ vs. VSMOW for $\delta^{18}O$ (SO_4), respectively.

3. Results and discussion

3.1. Sulfate reduction

Sulfate reduction is a common phenomenon observed in Fe° based PRB's due to favorable growth environment i.e. close to neutral pH and a very low ORP conditions (Gu et al., 2002). The