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Rethinking anaerobic As(III) oxidation in filters: Effect of indigenous nitrate respirers

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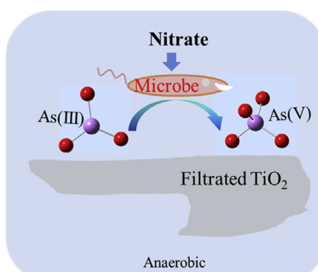
HIGHLIGHTS

- As(III) coexisting with indigenous groundwater particles was oxidized in the presence of nitrate.
- *Proteobacteria* dominated the groundwater microbe, with *Hydrogenophaga* (34%) as the major genera.
- Nitrate respirers especially from *Hydrogenophaga* anaerobically oxidized As(III).

GRAPHICAL ABSTRACT



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ABSTRACT

Microorganisms play a key role in the redox transformation of arsenic (As) in aquifers. In this study, the impact of indigenous bacteria, especially the prevailing nitrate respirers, on arsenite (As(III)) oxidation was explored during groundwater filtration using granular TiO_2 and subsequent spent TiO_2 anaerobic landfill. X-ray absorption near edge structure spectroscopy analysis showed As(III) oxidation (46% in 10 days) in the presence of nitrate in the simulated anaerobic landfills. Meanwhile, iron (Fe) species on the spent TiO_2 were dominated by amorphous ferric arsenate, ferrihydrite and goethite. The Fe phase showed no change during the anaerobic landfill incubation. Batch incubation experiments implied that the indigenous bacteria completely oxidized As(III) to arsenate (As(V)) in 10 days using nitrate as the terminal electron acceptor under anaerobic conditions. The bacterial community analysis indicated that various kinds of microbial species exist in groundwater matrix. Phylogenetic tree analysis revealed that *Proteobacteria* was the dominant phylum, with *Hydrogenophaga* (34%), *Limnohabitans* (16%), and *Simplicispira* (7%) as the major bacterial genera. The nitrate respirers especially from the *Hydrogenophaga* genus anaerobically oxidized As(III) using nitrate as an electron acceptor instead of oxygen. Our study implied that microbes can facilitate the groundwater As oxidation using nitrate on the adsorptive media.

1. Introduction

Various treatment technologies have been extensively studied during last decades to mitigate the severe problem of arsenic (As) tainted groundwater (Hu et al., 2015; Meng et al., 2001; Niazi et al.,

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2018; Niazi and Burton, 2016). As a consequence of enhanced As removal, a significant amount of As-bearing solid residuals is generated and disposed of in landfills, where As speciation and fate are generally determined by the ubiquitous microbes in the aquifer (Clancy et al., 2013; Jing et al., 2008).

A series of arsenite (As(III)) oxidizing bacteria have been successfully isolated and employed to transform As(III) to arsenate (As(V)) for As remediation (Lievremont et al., 2003; Wan et al., 2010), because As(V) is much more strongly adsorbed than As(III) (Pierce and Moore, 1982; Smedley and Kinniburgh, 2002). Our previous studies suggest that As(III) was oxidized to As(V) upon adsorption on TiO_2 in the filters, and As(V) was the primary As species in the effluent (Cui et al., 2015a). This oxidation process is often attributed to the marginally penetrated oxygen during filtration (Hu et al., 2015; Jing et al., 2009). Considering the ubiquitous existence of As(III) oxidizing bacteria in groundwater (Ghosh et al., 2014; Wang et al., 2014), we hypothesize that the indigenous As(III) oxidizing bacteria in groundwater should affect the speciation of adsorbed As in filters.

In the subsurface, As is often released into the groundwater coupled with a microbial-reductive process (Fendorf et al., 2010; Smedley and Kinniburgh, 2002). For instance, As remobilization may occur when As-bearing solid residuals from column filtration are landfilled (Jing et al., 2008). Under such anoxic conditions, microorganisms critically influence As release using common oxyanions including sulfate (Burton et al., 2011, 2014, 2013; Sun et al., 2016) and nitrate (Oremland et al., 2002; Rhine et al., 2006) as terminal electron accepters. A previous study has indicated that microbial sulfate reduction often leads to the formation of FeS, which sequestrated As by forming an As_2S_3 like complex on the mineral surface (Burton et al., 2014). While for nitrate, high concentrations often restrain the release of As (Harvey et al., 2002; Schaefer et al., 2016; Zhang et al., 2017a), which is attributed to the nitrate-dependent bacterial oxidation of As(III) (Senn and Hemond, 2002). A variety of bacterial isolates or communities can oxidize As(III) with nitrate as an electron acceptor under anaerobic conditions (Wang et al., 2017; Zhang et al., 2015, 2017b). However, the impact of indigenous nitrate respirers in groundwater on As(III) oxidation in filters and subsequent landfill of the As-laden materials remains unclear. Considering high nitrate concentrations in groundwater (up to 25–198 mg/L) due to agricultural activities (Jessen et al., 2017), the effect of nitrate and indigenous nitrate respirers on As speciation in filters and landfills motivated our study.

The objective of this study was to investigate the role of indigenous bacteria and nitrate on the transformation of As during groundwater filtration and in landfills. The change in As and Fe speciation on the spent $GTiO_2$ under anaerobic conditions was studied using X-ray absorption near edge structure (XANES) spectroscopy. The microbial diversity of the indigenous bacteria was characterized using 16S rRNA sequences. By combining XANES and 16S rRNA analysis, we intend to elucidate the effect of microbe and nitrate supplementation on As transformation. The result of our study will improve the understanding of the fate of As during filtration and anaerobic landfills.

2. Materials and methods

2.1. Materials

Stock solutions were prepared with reagent grade chemicals from Sinopharm Chemical Reagent Co. Ltd., China. $GTiO_2$ was synthesized using our previous method (Cui et al., 2015a). The particle size of $GTiO_2$ was 60–80 mesh, with a surface area of 196 m^2/g and a point of zero charge at 5.8 (Hu et al., 2015). Geogenic groundwater (GGW) from a rural well containing 642.0 ± 28.2 μg/L As(III) and 89.4 ± 9.9 μg/L As(V) with a pH value at 8.20 ± 0.06 (Table S1) was used in $GTiO_2$ loaded columns on site in Shanxi, China (Cui et al., 2013). The synthetic groundwater (SGW, Table S1) was prepared using boiled deionized (DI) water with N_2 purging to expel dissolved oxygen.

2.2. Filtration experiments

Field filtration experiments were conducted on site at Shanxi, China (N 39.43781, E 112.93448). Approximately 14.6 g $GTiO_2$ were loaded in a 1.2-cm diameter column, resulting in a bed volume (BV) of 14.5 mL. The empty bed contact time (EBCT) for the filter was determined according to the following equation: $EBCT = V_{empty}/Q$, where Q is the water flow rate (mL/min), and V_{empty} is the volume of the empty bed. The designed EBCT was 0.21 min controlled using a peristaltic pump (BT00-300 M, Longer, China).

Three treatments of $GTiO_2$ columns in duplicates were (1) GGW, (2) GGW amended with 10 mg/L nitrate, and (3) SGW. The influent and effluent were collected periodically, passed through a 0.22 μm membrane filter, and then preserved in the dark with 5% HCl at 4 °C before analysis. At the end of a filtration cycle, the columns were stored on dry ice in a cooler in the field and transported to the lab.

Once arrived in the lab, one duplicate of the columns with spent $GTiO_2$ and residual water was preserved under the anaerobic conditions (glovebox with 100% N_2) simulating the landfill disposal. The spent $GTiO_2$ preserved for 0, 4, and 10 d were sampled for XANES analysis.

The indigenous microorganisms were obtained by extracting the spent $GTiO_2$. Specifically, the spent $GTiO_2$ from the other duplicate column was mixed with 50 mL DI water for 15 min, and then the suspension was passed through a 0.22 μm autoclaved membrane under anaerobic conditions. The retained particles on the membrane filters were considered to be the groundwater matrix bearing indigenous microorganisms (Das et al., 2013; Li et al., 2013) for incubation experiments. The obtained indigenous microbes were preserved at −80 °C before bacterial community characterization.

2.3. Anaerobic incubation with indigenous microorganisms

To study microbial As(III) oxidation, the indigenous microorganisms were mixed with SGW containing 1.8 mg/L As(III) and 10.0 mg/L nitrate under anaerobic incubations (glovebox with 100% N_2). For comparison, incubation was also performed with no addition of nitrate. To test the role of microbes, the particles extracted from the spent $GTiO_2$ were autoclaved and added into the incubation experiment as a control study.

2.4. Bacterial community analysis

Bacterial DNA was extracted from the indigenous particles from the spent $GTiO_2$ using a Power Soil DNA kit (Mo Bio Laboratories, Carlsbad, CA, USA). The extracted DNA was purified using an AxyPrep polymerase chain reaction (PCR) Cleanup Kit (Axygen, USA). The 16S rRNA gene was amplified using universal primers, 27F (50-AGAGTTTGATCCTGGCT-CAG-30) and 1492R (50 -GGTTACCTTGT-TACGACTT-30) (Moreno et al., 2002). Standard procedures were used for ligation and DNA cloning. Approximately 100 white colonies were chosen in the PCR-restriction fragment length polymorphism (RFLP) analysis. The colony PCR products were digested separately with 2.5 U of *Msp*I and *Hin6*I in a 20 μL reaction mixture. Representative clones of each RFLP group were sequenced, giving 44 non-repetitive sequenced clones. These sequences were submitted to the NCBI (National Center for Biotechnology Information)