Water Research 141 (2018) 289–296

Contents lists available at ScienceDirect

Water Research

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

Synchronous microbial vanadium (V) reduction and denitrification in groundwater using hydrogen as the sole electron donor

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ARTICLE INFO

Article history:
Received 19 February 2018
Received in revised form
18 May 2018
Accepted 18 May 2018
Available online 21 May 2018

Keywords:
Vanadium (V)
Hydrogen
Nitrate
Bio-reduction
Denitrification

ABSTRACT

Groundwater co-contaminated by vanadium (V) (V(V)) and nitrate requires efficient remediation to prevent adverse environmental impacts. However, little is known about simultaneous bio-reductions of V(V) and nitrate supported by gaseous electron donors in aquifers. This study is among the first to examine microbial V(V) reduction and denitrification with hydrogen as the sole electron donor. V(V) removal efficiency of 91.0 ± 3.2% was achieved in test bioreactors within 7 d, with synchronous, complete removal of nitrate. V(V) was reduced to V(IV), which precipitated naturally under near-neutral conditions, and nitrate tended to be converted to nitrogen, both of which processes helped to purify the groundwater. Volatile fatty acids (VFAs) were produced from hydrogen oxidation. High-throughput 16S rRNA gene sequencing and metagenomic analyses revealed the evolutionary behavior of microbial communities and functional genes. The genera *Dechloromonas* and *Hydrogenophaga* promoted bio-reductions of V(V) and nitrate directly coupled to hydrogen oxidation. Enriched *Geobacter* and denitrifiers also indicated synergistic mechanism, with VFAs acting as organic carbon sources for heterotrophically functional bacteria while reducing V(V) and nitrate. These findings are likely to be useful in revealing biogeochemical fates of V(V) and nitrate in aquifer and developing technology for removing them simultaneously from groundwater.

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1. Introduction

Vanadium is a transition metal prevalent in the Earth's crust and is extensively used in modern technologies (Naeem et al., 2007; Sturini et al., 2013; Cao et al., 2017). Geological weathering and discharges from industrial processes lead to the presence of vanadium in groundwater (Chen and Liu, 2017). In the U.S., substantial vanadium contamination has been recorded at 283 superfund sites, with vanadium concentration reaching 100 μM in an aquifer at a site near Rifle, Colorado (Yelton et al., 2013), far exceeding the 0.2 μg/L minimum reporting level proposed by the U.S. Environmental Protection Agency. In China, groundwater at Panzhihua, famous for its vanadium titanomagnetite resources and vanadium production, also contains very high concentrations of vanadium, up to 0.2 mg/L (Liu et al., 2017). Vanadium is moderately poisonous, with its toxicity increasing with valence state and solubility (Ortiz-Bernad et al., 2004; Zhang et al., 2010). Vanadium (V) (V(V)) is the most toxic and mobile form, whereas vanadium (IV) (V(IV)) is less toxic and insoluble at near-neutral pH (Safavi et al., 2000; Wang and Ren, 2014). Over recent decades, nitrate pollution of groundwater has become a serious issue worldwide owing to the increasing use of nitrogenous fertilizers, and discharges of domestic and industrial wastewater (Zhang et al., 2014a; Xie et al., 2018). Excess nitrate in drinking water poses health risks, including gastric problems in adults, decreased functioning of the thyroid gland, and multiple sclerosis (Cai et al., 2015; Zhai et al., 2017). Groundwater co-contaminated by V(V) and nitrate is increasingly commonplace, such as in the Gulf Coast aquifer of Texas, with concentrations of both contaminants exceeding established maximum levels or health risk limits (Glenn and James Lester, 2010).

Reduction of V(V) to V (IV) is the approach usually taken to remove vanadium from contaminated groundwater (Zhang et al., 2015). Physical and chemical methods are commonly used for

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https://doi.org/10.1016/j.watres.2018.05.033
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V(V) removal (Reul et al., 1999), but their cost-effectiveness is questionable, and secondary pollution may also occur. Microbial V(V) reduction is increasingly recognized as a promising future strategy for remediation of V(V) contaminated groundwater, due to its simplicity, sustainability, and low cost (Lovley and Coates, 1997; Yelton et al., 2013). Various microorganisms, such as *Geobacter metallireducens*, *Shewanella oneidensis*, and *methanogens*, which naturally occur in groundwater, are capable of reducing V(V) to V(IV) (Liu et al., 2016). Most known species are heterotrophic and consume organic carbon; however available organics decrease with increasing depth in the subsurface geological environment (Singh et al., 2015). Supplementary addition of soluble organics can increase remediation costs and bring about potentially secondary pollution. Aquifer clogging may also take place due to higher biomass production of heterotrophs (Li et al., 2010). Thereby autotrophic bioremediation with inorganic electron donors is of particular importance. Furthermore, aquifers may contain dissolved hydrogen due to the hydration and oxidation of rocks and minerals (Miller et al., 2017). As an electron donor, hydrogen possesses several advantages in that it is inexpensive, widely available, and non-toxic (Rittmann et al., 2004). As a result hydrogen has been successfully employed to support bio-reductions of contaminants in groundwater, including chromate (Singh et al., 2015), arsenate (Chung et al., 2006), selenate (Van Ginkel et al., 2011), perchlorate and nitrate (Zhao et al., 2013). To date, limited studies have focused on hydrogen-based microbial V(V) reduction (Xu et al., 2015), and the interactions between V(V) and other co-contaminants in bioreduction processes remain unknown.

Herein, we investigate experimentally the bioremediation of V(V) and nitrate co-contaminated groundwater with hydrogen as the sole electron donor. The paper aims to explore the simultaneous removal of V(V) and nitrate which often occur together in groundwater, and to reveal dynamics of microbial communities, dominant species, and functional genes.

2. Materials and methods

2.1. Experimental setup and operation

Eight cubic bioreactors were employed, made of plexiglass with total volume of 280 mL and covered with aluminum foil. Two holes were located in the top of the reactor, one for replacing the culture medium and for sampling, and the other for hydrogen injection. Each bioreactor was filled with synthetic groundwater containing the following mineral salts per L: $CaCl_2$ 0.2464 g, $MgCl_2 \cdot 6H_2O$ 1.0572 g, NaCl 0.4459 g, KCl 0.0283 g, $NaHCO_3$ 0.504 g, and KH_2PO_4 0.0299 g. V(V) was provided in the form of $NaVO_3$ at a prescribed concentration. Each bioreactor was inoculated with 20 mL anaerobic sludge extracted from an upflow anaerobic sludge blanket reactor used to treat high strength wastewater (Beijing YanJing Brewery Co. Ltd, China). The sludge shared the similar microbial community structure with groundwater microbes in vanadium contaminated aquifer (Cao et al., 2017). They were divided into four groups: BR-V-N comprising medium containing both 1 mM V(V) and 1 mM nitrate with hydrogen donor; BR-V consisting solely of 1 mM V(V), again with hydrogen donor; and BR-N consisting solely of 1 mM nitrate, with hydrogen donor; and a control group BR comprising medium containing both 1 mM V(V) and 1 mM nitrate without hydrogen donor. In all cases, except BR, air in the headspace of all bioreactors (30 mL) was first expelled, and then hydrogen was injected via a syringe, after which the bioreactors were sealed with a rubber plug. A further two reactors without inoculation but with hydrogen in the headspace were assigned as AR, and filled with same medium as BR-V-N.

The inoculated bioreactors were first incubated for 3 months, refreshing the aqueous solution every 7 days. Soluble organics existing in the inocula originally were almost depleted after cultivation. After that the feasibility of simultaneous removal of V(V) and nitrate was evaluated by employing hydrogen as the sole electron donor in three consecutive operating cycles (each lasting 7 d), and comparing the resulting concentrations against those in the control tests. Consumption of hydrogen and generation of gaseous products during this process were examined by collecting samples from the headspace into airbags for measurement. Soluble and solid products were also analyzed. A parameter study was then undertaken whereby the influence of key operating factors on hydrogen-supported V(V) reduction and denitrification was examined for different initial nitrate concentrations (0.5 mM, 1 mM and 1.5 mM) with fixed 1 mM V(V), and different V(V) loadings (0.5 mM, 1 mM and 1.5 mM) with fixed mole ratio of V(V) and nitrate (1:1). Liquid samples were acquired by sterilized syringes at selected time intervals, and the samples filtered immediately through a 0.22-μm polyether sulphone membrane filter for analysis. Each time after sampling, hydrogen was replenished through a needle connecting with hydrogen cylinder at the rate of 100 mL/min for 30 min to supply sufficient electron donor. Then another 3 months accumulation was conducted for BR-V-N for high-throughput sequencing analysis. For each sample, microbial community analysis was carried out in triplicate to confirm the reproducibility. The two reactors in each group were operated under identical conditions, and the mean results recorded. All experiments were conducted at room temperature (22 ± 2 °C).

2.2. Analytical methods

The presence of V(V) was analyzed using spectrophotometry (Zhang et al., 2012). Total V was analyzed using inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Fisher X series, Germany). Nitrate, nitrite and ammonium were monitored using a spectrophotometer (DR6000, HACH, the USA). Total organic carbon (TOC) was measured by Multi N/C 3000 TOC analyzer (Analytik Jena AG, Germany). Gas chromatograph (Agilent, 4890, J&W Scientific, USA) was employed to analyze gases, including hydrogen and nitrogen by means of a thermal conductivity detector, and volatile fatty acids (VFAs) using a flame ionization detector. Precipitates that appeared during the operation were collected through centrifugation at 10,000 rpm and analyzed using X-ray photoelectron spectroscopy (XPS) (XSAM-800, Kratos, UK).

2.3. Microbiological analysis

Biomass in BR-V-N and the originally inoculated sludge underwent ultrasonic pretreatment, and their total genomic DNA was extracted using a FastDNA® SPIN Kit (Qiagen, CA, the USA), following manufacturer's instructions. Then the above DNA was amplified with PCR primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). A mixture of amplicons was used for high-throughput 16S rRNA gene and metagenomics analyses using MiSeq (Illumina, USA), performed by Shanghai Majorbio Technology (Shanghai, China). Raw data were submitted to the public National Center for Biotechnology Information (NCBI) database with accession numbers: SRP096812 and SRP120206. Operational taxonomic units (OTUs) were clustered from sequences by setting a 0.03 distance limit (equivalent to 97% similarity). Rarefaction curves and alpha diversity indexes were obtained using Mothur (version v.1.30.1). Phylogenetic affiliations and metagenomic results were analyzed using the RDP Classifier by comparison with the silva (SSU115) 16S rRNA database and Kyoto Encyclopedia of Genes and Genomes (KEGG) database, following previous studies (Lai et al., 2016).

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