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

Yufeng Jiang^a, Baogang Zhang^{a*}, Chao He^a, Jiaxin Shi^a, Alistair G.L. Borthwick^b,

Xueyang Huang^a

*^a School of Water Resources and Environment, MOE Key Laboratory of Groundwater
Circulation and Environmental Evolution, China University of Geosciences (Beijing),
Beijing 100083, P. R. China*

*^b School of Engineering, The University of Edinburgh, The King's Buildings,
Edinburgh EH9 3JL, UK*

*Corresponding author. Tel.: +86 10 8232 2281; Fax: +86 10 8232 1081.

E-mail: zbgcugb@gmail.com, baogangzhang@cugb.edu.cn (B. Zhang).

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 $91.0 \pm 3.2\%$ 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.**

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

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 $\mu\text{g/L}$ 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., 2016). 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 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 bio-reduction 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: CaCl₂ 0.2464 g, MgCl₂·6H₂O 1.0572 g, NaCl 0.4459 g, KCl 0.0283 g, NaHCO₃ 0.504 g, and KH₂PO₄ 0.0299 g. V(V) was provided in the form of NaVO₃ 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- μ m 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).

3. Results and discussion

3.1. Hydrogen-supported V(V) and nitrate removal

Both V(V) and nitrate gradually decreased in BR-V-N over three consecutive operating cycles (Fig. 1a), during which simultaneous removal of V(V) and nitrate

took place, with a V(V) removal efficiency of $91.0 \pm 3.2\%$ ($p < 0.05$), and complete removal of nitrate within 7 d operation. The average removal rates were 124.7 ± 2.6 $\mu\text{M/d}$ for V(V) and 173.6 ± 3.9 $\mu\text{M/d}$ for nitrate ($p < 0.05$) (Fig. 1b), with pseudo first-order rate constants of 0.2601 d^{-1} and 0.7252 d^{-1} (Supplementary information (SI), Table S1). To the authors' knowledge, this is the first report concerning the bio-reduction of V(V) and nitrate as co-contaminants, using hydrogen as the sole electron donor. The present research appears to be an improvement over existing studies to hydrogen-based V(V) bio-reductions in that the V(V) removal efficiency in 350 h was 95.5% for initial 2 mg/L V(V) and the calculated removal rate was only 0.005 mg/L·h by autohydrogentrophic bacteria (Xu et al., 2015), whereas 30 d was required to reduce 2 mM V(V) by mesophilic and thermophilic methanogens with H_2/CO_2 (80:20 gas mix) (Zhang et al., 2014b). The obtained V(V) removal rate was lower than that of 24.2 ± 1.7 $\mu\text{M/h}$ in the presence of nitrate and acetate due to differences in electron donors and carbon sources (Liu et al., 2017). V(V) was removed more slowly than nitrate in BR-V-N because of its higher toxicity to microbes (Liu et al., 2017).

Compared with removals of V(V) in BR-V and nitrate in BR-N (Fig. 1a), both V(V) and nitrate bio-reductions were suppressed in BR-V-N, due to the competitive consumption of electron donor. This phenomenon differed from result obtained using soluble organic carbon sources, where the bio-reduction of V(V) was accelerated by addition of chromium (VI), which is more toxic (Wang et al., 2017). Nitrate was removed relatively more slowly in the present study due to the discontinuous supply

of hydrogen (Wu et al., 2018). Furthermore, concentrations of V(V) and nitrate remained almost unchanged in AR (SI Fig. S1), which proved that V(V) and nitrate removal in BR-V-N was biologically mediated. Hardly any V(V) was removed in BR, with slight removal of nitrate (SI Fig. S1), implying that hydrogen played a key role by providing electrons for microbes to detoxify V(V) and nitrate.

3.2. Reaction products identification and process elucidation

Gradually decreasing total V was observed in a typical operating cycle (7 d) in BR-V-N (Fig. 2a), with appearance of blue precipitates, indicating the less mobile characteristic of the generated products. V 2p spectra of XPS were recorded for the precipitates (Fig. 2b). The sub-band with a peak at the binding energy of 515.9 eV was identified as V(IV) (Zhao et al., 2016; Zhang et al., 2018), providing direct evidence that V(V) was bio-reduced to less toxic V(IV). *In situ*, V(IV) can precipitate naturally in near-neutral conditions in the form of VO(OH)₂ and/or vanadyl phosphate [CaV₂(PO₄)₂(OH)₄·3H₂O] (Qiu et al., 2017). Peaks corresponding to V(V) were also observed, most likely due to the re-oxidation of generated V(IV) during the collection and testing of precipitates (Zhang et al., 2009a). As hydrogen was consumed, its content declined during successive sampling intervals (Fig. 2a). The hydrogen-based V(V) bio-reduction could therefore be elucidated by the following stoichiometric equation,

Meanwhile, nitrite and ammonium initially accumulated in BR-V-N (Fig. 2c),

indicating the presence of multiple metabolic pathways, including denitrification and dissimilatory nitrate reduction to ammonium (Tong et al., 2013). Both nitrite and ammonium gradually disappeared, perhaps due to the occurrence of anammox activities. Nitrogen accumulated in the headspace of the reactor during operation (Fig. 2a), and so hydrogen-based denitrification could be expressed as follows,

Mass balance and electron transfer were investigated based on the above equations. During a typical operating cycle (7 d), 11.36 ± 0.04 mmol electrons were released from hydrogen oxidation. The theoretically required amounts of electrons for removal of V(V) and nitrate were 0.25 ± 0.01 mmol and 1.40 ± 0.01 mmol ($p < 0.05$), along with the detected terminal reduction products. Hence, $2.20 \pm 0.03\%$ and $12.32 \pm 0.07\%$ ($p < 0.05$) of released electrons were consumed for V(V) respiration and denitrification. The remaining electrons contributed to microbial growth and proliferation. It should be noted that the utilization of hydrogen could be further improved by diffusing hydrogen through the gas transfer membrane (Wu et al., 2018). When practical bioremediation was conducted, the insufficiency of hydrogen could be compensated by supplying hydrogen continuously through sustainable hydrogen production, such as via bioelectrochemical systems (Zou and He, 2018). Detoxification of V(V) and nitrate could be realized by bioaugmentation through stimulating indigenous microorganisms in aquifer by hydrogen. According to Equ. (2), the theoretical nitrogen yield was 0.13 ± 0.02 mmol, while the monitored nitrogen was 0.11 ± 0.03 mmol in 7 d operation; this was probably due to the generation of

other nitrogen-containing intermediates and assimilations (Zhang et al., 2014a). Hydrogen was found in aquifers with concentration around 15 nM (Chapelle et al., 1996), thus results from this study could be helpful to reveal the biogeochemical fates of V(V) and nitrate in groundwater. Lower biomass yields were produced in hydrogen-based bioprocess (Wu et al., 2017), thus possible clogging of aquifer could be prevented when it was practically applied.

Residual VFAs were also detected during the operation of BR-V-N (Fig. 2d), with average concentration of 9.84 ± 1.56 mg/L in a 7 d operating cycle ($p < 0.05$), comparable with result from methane-based biological bromate reduction (Luo et al., 2017). These metabolites might be derived from hydrogen oxidation and bicarbonate reduction by autotrophic microbes with the function of hydrogenases encoded by genes such as *hhyL* gene (Khdhiri et al., 2015). They could be consumed by heterotrophic microbes for V(V) and nitrate bio-reductions (Lai et al., 2016). Unlike acetate, which was the main form of residual VFAs under quasi-anaerobic condition (Luo et al., 2018), valeric species were the dominant components in our study, probably due to the immediate consumption of other species after their synthesis by heterotrophic microbes. For example, microorganisms were favor to using acetate as carbon source during V(V) bio-reduction (Liu et al., 2016). Less VFAs were accumulated with average TOC concentration in a typical operating cycle of 1.26 ± 0.11 mg/L ($p < 0.01$), which was much lower than original TOC concentration in natural groundwater (around 10 mg/L) (Zhang et al., 2017a). This result suggested that the VFAs residue could hardly cause significant fluctuations of organics

concentration when this hydrogen-based process was performed.

3.3. Influences of operating factors

Both V(V) and nitrate in BR-V-N decreased progressively with time, with lower values observed at any given time for lower initial nitrate concentrations (Fig. 3a). Removal efficiencies of V(V) reached $99.2 \pm 0.9\%$, $91.0 \pm 3.2\%$ and $78.6 \pm 4.2\%$ after 7 d for initial nitrate concentrations of 0.5 mM, 1 mM and 1.5 mM ($p < 0.05$). The corresponding pseudo first-order rate constants were 0.3561 d^{-1} , 0.2603 d^{-1} and 0.1921 d^{-1} (SI Fig. S2a, Table S2). Nitrate was removed more quickly than V(V) under each test condition (Fig. 3b), with pseudo first-order rate constants of 0.9391 d^{-1} , 0.7245 d^{-1} and 0.6035 d^{-1} . In practice, V(V) and nitrate compete with respect to electron donor consumption, whereas nitrate is less toxic to microbes than V(V). Larger amounts of nitrate are commonly present in actual aquifers (Degnan et al., 2016), and so the interaction of nitrate with V(V) should be considered whenever practical bioremediation is conducted with hydrogen as the sole electron donor.

Both V(V) and nitrate were progressively removed with time under different V(V) loadings (Fig. 3c). Here V(V) removal efficiencies decreased as the V(V) loading increased, ranging from complete removal at a V(V) loading of 0.5 mM to $71.2 \pm 1.3\%$ at 1.5 mM V(V) loading ($p < 0.05$), with corresponding pseudo first-order rate constants of 1.5372 d^{-1} to 0.1882 d^{-1} (SI Fig. S2b, Table S2). Nitrate removal was faster than V(V) (Fig. 3d), with pseudo first-order rate constants of 1.6095 d^{-1} , 0.7252 d^{-1} and 0.5661 d^{-1} at V(V) loadings of 0.5 mM, 1 mM and 1.5 mM, respectively. V(V) and nitrate vary in aquifers (Schlesinger et al., 2017; Nolan and

Weber, 2015) and so the present results suggest that hydrogen-based bio-reductions of V(V) and nitrate can be feasible over a large range of V(V) loadings.

3.4. Microbial communities and mechanisms

Fewer OTUs were detected in the biomass from BR-V-N, compared with inoculated sludge (SI Fig. S3). This implies that the microbial richness decreased significantly due to the presence of V(V) and nitrate, which was also reflected by the Chao1 and Ace indexes (SI Table S3). The lower Shannon index and higher Simpson index suggested lower diversity and evenness in BR-V-N, indicating that the microbes were highly selected. Moreover, the microbial abundance within the hydrogen-fed autotrophic bioreactor was lower than that in heterotrophic systems for V(V) bio-reductions (Liu et al., 2016), most likely because of the limited metabolic pathways.

Evolution of different microbial communities was observed in BR-V-N at class level (Fig. 4a). *Spirochaetes* increased dramatically from $1.72 \pm 0.02\%$ in the inocula to $47.9 \pm 7.2\%$ and became dominant in BR-V-N ($p < 0.05$). Microbes in this class are capable of fermenting carbohydrates into simple organic acids (Sun et al., 2010), a process closely akin to nitrate reduction (Xu et al., 2017). *Bacteroidetes* BD2-2, *Betaproteobacteria*, *Actinobacteria* and *Mollicutes* were also noticeably enriched after cultivation in BR-V-N. Notably, *Betaproteobacteria* and *Actinobacteria* species are associated with dissimilatory metal reductions and denitrification (Hao et al., 2016; Cesarano et al., 2017).

Functional species were found at genus level in BR-V-N (Fig. 4b). Primary

examples included the genera *Dechloromonas* and *Hydrogenophaga* which are reported to be autohydrogenotrophic denitrifiers (Zhang et al., 2009b; Zhang et al., 2017b) and *Geobacter* which promotes the reduction of V(V) (Ortiz-Bernad et al., 2004). Enriched *Nitrospira*, *Ideonella* and *Methyloversatilis* took part in nitrogen cycling (Shi et al., 2017; Pepe-Rannek et al., 2015; Baytshtok et al., 2009). The gathered denitrifiers could be also responsible for V(V) removal given that V(V) bio-reductions shared similar reductases with denitrification (Xu et al., 2015). Interestingly, Fe(III) and sulfate-reducing bacteria also accumulated, including *Sphaerochaeta* and *Desulfuromonas* (Ritalahti et al., 2012).

Metagenomic analysis with functional genes and encoding proteins summarized into eight catalogs revealed the microbial dynamics at molecular level (Fig. 4c). Details were listed in SI Table S4 and Table S5. Metal reduction genes and their corresponding proteins increased significantly in BR-V-N, confirming the resultant V(V) bio-reductions through pathways presented in SI Table S6. Similar tendencies were found in denitrification genes and the corresponding enzymes, consistent with the detected denitrifiers. NADP genes related to hydrogen metabolism bloomed due to the presence of hydrogen. Although genes related to phosphate transporters decreased, their corresponding proteins were enriched, which favored V(V) removal, noting that ABC transporters have been reported to be linked to selenate bio-reduction (Lai et al., 2016). Notably, genes responsible for the synthesis of electron shuttles (flavodoxin, ubiquinone, cytochrome, etc) also increased, facilitating electron transfers from electron donor (hydrogen) to electron acceptors (V(V) and nitrate) (Kranz-Finger et

al., 2018).

Statistical studies revealed correlations between representative accumulated functional genes and microbes (Fig. 4d). Genera *Dechloromonas*, *Ideonella* and *Methyloversatilis* showed strongly positive relationships with genes involved in metabolic pathways, microbial metabolism in diverse environments, biosynthesis of secondary metabolites, and nitrogen metabolism such as *sdhA*, *nifJ*, *gltD* and *narG* (Guardia et al., 2018). Genera *Hydrogenophaga* and *Geobacter* were linked with the *chrA* gene, which participated in metal reductions (Shaw and Dussan, 2018). Genus *Desulfuromonas* was positively related to *phsA* and *cysH* genes, which took part in the removal of nitrite and metal (Haveman et al., 2004; Saltikov and Newman, 2003).

The mechanisms of hydrogen-based V(V) bio-reduction and denitrification were now summarized. Both direct and synergistic routes were proposed (Fig. 5). Detection of *Dechloromonas* and *Hydrogenophaga* proved that hydrogen oxidation could be coupled directly to nitrate removal. V(V) bio-reduction could also be performed by these species given that their membrane-bound nitrate reductases had been implicated in V(V) respiration (Xu et al., 2015). Through synergy, hydrogen was consumed by hydrogen-utilizing bacteria such as *Bacillus* to synthesize VFAs (Chubukov and Sauer, 2014), after which the generated VFAs served as electron donors and carbon sources for the heterotrophic V(V)-reducing bacterium *Geobacter* involved in V(V) bio-reduction, and denitrifiers for nitrate removal. Functional genes such as *gltD*, *chrA* and *narG* encoded corresponding enzymes that conducted simultaneous V(V) bio-reduction and denitrification coupled to hydrogen oxidation.

348

349 **4. Conclusions**

350 In this study, co-contaminants V(V) and nitrate in groundwater were handled in
351 anaerobic bioreactors. The major conclusions and outcomes are:

- 352 • Synchronous V(V) bio-reduction and denitrification can be realized with
353 hydrogen as the sole electron donor.
- 354 • V(V) removal efficiency of $91.0 \pm 3.2\%$ was achieved in a typical operating
355 cycle (7 d), along with complete removal of nitrate.
- 356 • V(IV) formed as the reduction product of V(V), and nitrate was reduced to
357 nitrogen.
- 358 • The dynamics of microbial communities and functional genes provide
359 evidence for both direct and synergetic mechanisms.

360

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Figure captions.

Fig. 1. Temporal variations in V(V) and nitrate in hydrogen-based bioreactors. (a) time histories of V(V) and nitrate concentrations during three consecutive operating cycles; (b) pseudo first-order kinetic plots for a typical operating cycle (7 d). Red arrows indicate the replacement of synthetic groundwater. C_0 (μM) and C_t (μM) are the concentrations of V(V) or nitrate at initial condition and at time t (d), respectively.

Fig. 2. Reaction products during the operation of BR-V-N. (a) variation in total V, hydrogen, and nitrogen with time; (b) XPS spectra of V 2p for the generated precipitates; (c) variation in nitrite and ammonium with time; (d) average concentrations of residual VFAs.

Fig. 3. Parameter study of operating factors affecting the performance of BR-V-N. (a) V(V) and (b) nitrate removal time histories for different initial nitrate concentrations; (c) V(V) and (d) nitrate bio-reduction time histories for different V(V) loadings.

Fig. 4. Microbial community compositions and functional genes revealed by sequencing of inoculated sludge and BR-V-N. (a) class-level abundance; (b) functional bacterial genera; (c) relative abundance of functional genes and proteins; (d) heatmap of representative functional genes based on bacterial genera.

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