Enhancement of synchronous bio-reductions of vanadium (V) and chromium (VI) by mixed anaerobic culture☆

Song Wang [a], Baogang Zhang [a, *], Muhe Diao [b], Jiaxin Shi [a], Yufeng Jiang [a], Yutong Cheng [a], Hui Liu [a]

[a] School of Water Resources and Environment, MOE Key Laboratory of Groundwater Circulation and Environmental Evolution, China University of Geosciences (Beijing), Beijing 100083, PR China
[b] Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, 1090 GE, Amsterdam, the Netherlands

ARTICLE INFO

ABSTRACT

The co-occurrence of toxic vanadium (V) and chromium (VI) in groundwater receives incremental attention while knowledge on their interactions in biogeochemical processes is limited, with lack of efficient removal means. This study is the first to realize synchronous bio-reductions of V(V) and Cr(VI) with high efficiency by mixed anaerobic culture. After 72-h operation, $97.0 \pm 1.0\%$ of V(V) and $99.1 \pm 0.7\%$ of Cr(VI) were removed, respectively, with initial concentration of 1 mM for both V(V) and Cr(VI). Cr(VI) bio-reduction took priority while V(V) detoxification was inhibited. V(IV) and Cr(III) were the identified reduction products, both of which could precipitate naturally. Initial Cr(VI) and acetate concentrations as well as pH affected this process significantly. High-throughput 16S rRNA gene sequencing analysis indicated the accumulation of *Anaerolineaceae*, *Spirochaeta* and *Spirochaetaceae*, which could contribute to V(V) and Cr(VI) bio-reductions. The new knowledge obtained in this study will facilitate understanding the biogeochemical fate of co-existing V(V) and Cr(VI) in groundwater and development of bioremediation strategy for their induced combined pollution.

1. Introduction

Combined pollution, especially occurs during intensive mining and smelting activities, brings serious threat to geological environment (Zhang et al., 2012; Liu et al., 2015). Vanadium and chromium often symbiotically exist in minerals, such as vanadium bearing titanomagnetite in China (Wang et al., 2017b). In the processing of these ores, large amounts of dusts and wastewater containing vanadium (V) (V(V)) and chromium (VI) (Cr(VI)) are produced, which may enter soil and groundwater (Fang et al., 2017; Cao et al., 2017). Groundwater co-contaminated by V(V) and Cr(VI) is gradually reported world-wide (Mitchell et al., 2011; Rasheed et al., 2012). Though biogeochemical processes of vanadium and chromium in soil are revealed (Shaheen et al., 2014a, 2014b), knowledge is limited on their biogeochemical cycling in groundwater as well as their interaction during this process. Toxicities of vanadium and chromium increase with their valence states and

solubilities, while V(V) and Cr(VI) are the most toxic (Mochizuki et al., 1999; Yelton et al., 2013; Narayani and Shetty, 2013; Yang et al., 2017). Besides commonly employed adsorption by various adsorbents to remove heavy metals and organics from water environment (Saleh et al., 2017a, 2018; Altıntıg et al., 2017), reduction of V(V) and Cr(VI) to less toxic and mobile V(IV) and Cr(III), respectively, are recognized as a reasonable route to detoxify them in groundwater (Ortiz-Bernad et al., 2004; Chebeir et al., 2016; Chen and Liu, 2017; Liu et al., 2017).

Chemical reductions have been investigated to conduct these transformations, but their cost-effectiveness and potential secondary pollution must be further addressed (Reul et al., 1999; Yurkow et al., 2002). Biological treatment based on anaerobic microbial V(V) and Cr(VI) reductions appears to be a more promising approach because of its low cost and potential applications for *in situ* remediation (Li et al., 2007; Reijonen et al., 2016), with discoveries of microbes that can reduce V(V) and Cr(VI) such as *Geobacter metallireducens* and *Shewanella oneidensis* (Ortiz-Bernad et al., 2004; Wang et al., 2017a). However, most existing studies handle V(V) or Cr(VI) individually by pure or mix cultures (Hao et al., 2015; Singh et al., 2015; Zhang et al., 2014; Gong et al.,

---

2018), with mixed cultures possessing higher microbial diversity, great adaption, self-evolution abilities and higher reduction efficiency (Zhang et al., 2015). Removals of these two metal ions have been investigated with the co-existence of other contaminants. For instance, V(V) bio-reduction is evaluated with the presence of nutrients (Jiang et al., 2018), and the effect of nitrate on microbial Cr(V) reduction is also studied (Zhong et al., 2017). Though principles of reducing V(V) and Cr(VI) in parallel by pure culture is revealed previously (Wang et al., 2017a), little is known on interactions of V(V) and Cr(VI) as well as successions of microbial communities during their bio-reductions by mixed anaerobic culture, which is just the case with groundwater (Cho et al., 2013).

Herein, we have experimentally investigated the microbial reduction process performed by mixed anaerobic culture with the presence of V(V) and Cr(VI) together. The specific research objectives are: (1) to understand the interactions of microbial V(V) and Cr(VI) reductions with examinations of reduction products and influences of operating factors; and (2) to reveal the microbial community evolutions and identify the dominant species involved in microbial V(V) and Cr(VI) reductions.

2. Materials and methods

2.1. Batch bioreactor setup and operation

Six 250-mL glass bottles with silica gel stoppers were used as batch bioreactors. Each bioreactor was filled with 50 mL anaerobic sludge collected from an up-flow anaerobic sludge blanket (UASB) reactor treating high strength wastewater (Beijing YanJing Brewery Co., LTD, China) and 200 mL synthetic groundwater containing following compounds per liter: NH_4Cl (0.1557 g), $CaCl_2$ (0.2464 g), $MgCl_2$ (1.0572 g), NaCl (0.4459 g), KCl (0.0283 g), $NaHCO_3$ (0.8082 g), and KH_2PO_4 (0.0299 g). The initial concentration of mixed liquor volatile suspended solids for the anaerobic sludge was $14.7 \pm 0.2\,g\,L^{-1}$. The synthetic groundwater was prepared based on deionized water with pH and conductivity of 8.36 and $3.67\,mS\,cm^{-1}$, respectively. Acetate was supplemented as organic carbon source, with the equivalent of $64\,mg\,L^{-1}$ chemical oxygen demand (COD) for per mM acetate (Liu et al., 2016). They were divided into three groups equally. One group which was added with both V(V) and Cr(VI) was designated as "compound system" (CS). The left two groups with V(V) or Cr(VI) separately were "single systems" (SS), which were designated as SS-V and SS-Cr, respectively. Another reactor inoculated with sterilized anaerobic sludge and fed with same solution as CS was designated as "blank system" (BS) for comparison. All chemicals were provided by Sinopharm Chemical Reagent Co., Ltd. (Beijing, China) and they were analytical grade without further purification. V(V) was added in the form of $NaVO_3$, and Cr(VI) was supplied as $K_2Cr_2O_7$.

All bioreactors were cultivated for 3 months to reach stable performance before beginning the formal experiments. Then their performances of V(V) and Cr(VI) removals were evaluated in three consecutive operating cycles (72 h for each cycle), with initial 1 mM V(V) and 1 mM Cr(VI) in CS, 1 mM V(V) in SS-V and 1 mM Cr(VI) in SS-Cr, respectively. Levels of V(V) and Cr(VI) concentrations were consistent with those employed previously for easy comparison (Liu et al., 2016; Wang et al., 2017a). Initial acetate concentration was fixed at 10 mM, with solution pH of 7. Experiments were also conducted in BS for comparison. The obtained data were fitted into the pseudo-first-order kinetics model to obtained removal rate constants. Variations of total V, total Cr and pH as well as acetate consumption were also systematically investigated in CS. Then influencing factors as initial Cr(VI) concentrations (0.5 mM, 1 mM, 1.5 mM, 2 mM), initial acetate concentrations (5 mM, 10 mM, 15 mM, 20 mM) and pH (6, 7, 8, 9) were examined individually in

CS. The consideration of initial Cr(VI) levels was that Cr(VI) with concentrations from dozens to hundreds milligram per liter was found in actually contaminated aquifer (Němeček et al., 2016; Huang et al., 2017). After that, biomass in all bioreactors was sampled for microbial analysis after another 3 months accumulation. All experiments were conducted in triplicates and at room temperature ($22 \pm 2\,°C$). The two bioreactors in each group were operated under identical condition and their mean values were reported. Statistical analyses were performed using SPSS 20 (SPSS Inc., Chicago, USA). The level for statistical significance was set at $p < 0.05$.

2.2. Analytical methods

Aqueous samples were filtered using cellulose acetate syringe filters with a pore size of 0.22-μm before analysis. Spectrophotometric methods were chosen to measure the concentration of V(V) and Cr(VI) by using a spectrophotometer (DR6000, HACH, the USA). 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP)
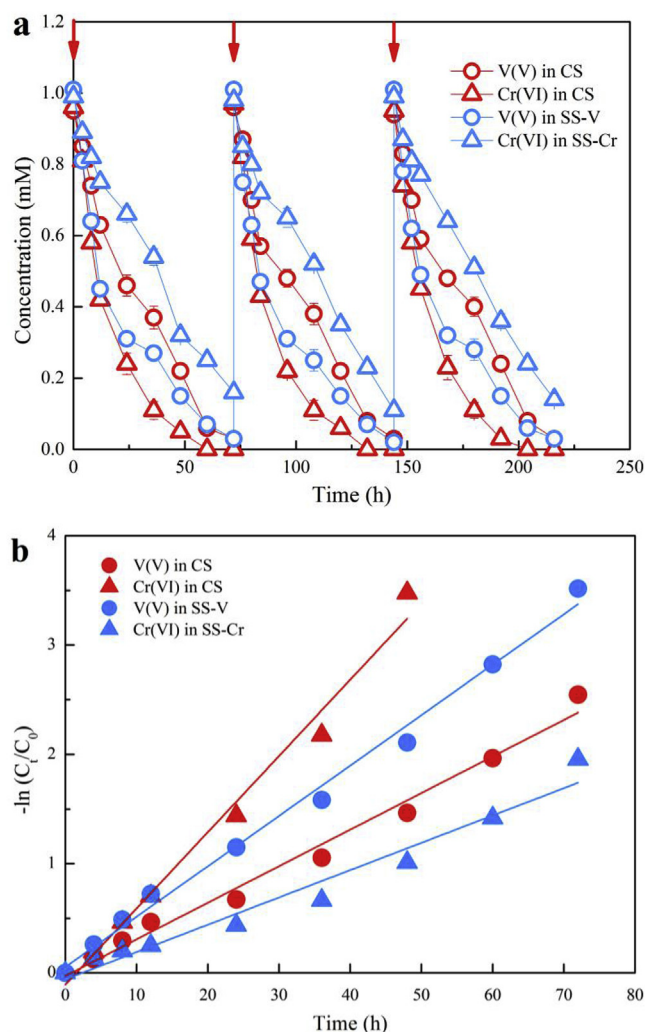

Fig. 1. Time courses of V(V) and Cr(VI) in employed bioreactors with kinetics analysis. (a) Changes of V(V) Cr(VI) in three consecutive cycles; (b) pseudo first-order kinetic plots in a typical cycle (72 h). Red arrows imply the replacement of synthetic groundwater. C_0 (μM) and C_t (μM) are the concentrations of V(V) or Cr(VI) at the initial condition and at time t (h), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was used as a chelating agent for determining V(V) at 603 nm (Deng et al., 2014), while Cr(VI) was analyzed by using a diphenyl carbazide method at 540 nm (Wang et al., 2018). COD was measured based on digestion with potassium dichromate in concentrated sulfuric acid (Zhang et al., 2009). pH was measured using a pH-201 m (Hanna, Italy). Total V and total Cr in aqueous solution were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Fisher X series, Germany). Generated precipitates in CS were collected by centrifugation, vacuum dried by a freeze-dryer (FD-1A-50, Shanghai Bilon, China) and examined by X-ray photoelectron spectroscopy (XPS) (XSAM-800, Kratos, UK) with Mg K$_\alpha$ radiation (13 kV, 20 mA).

2.3. Microbial analysis

Ultrasonic method was employed to collect biomass in bioreactors and inoculated mixed anaerobic culture. Their total genomic DNA was extracted with FastDNA® SPIN Kit for Soil (Qiagen, CA, the USA). The extracted DNA was then pooled and amplified by PCR (GeneAmp® 9700, ABI, the USA) with primers 338F (ACTCCTACGGGAGGC AGCAG) and 806R (GGACTACHVGGGTWTC-TAAT) (Cao et al., 2017). After that, the purified and quantified DNA was sent to Shanghai Majorbio Technology (Shanghai, China) for high-throughput 16S rRNA gene sequencing on MiSeq (Illumina, the USA). Sequences in this study had been submitted to the NCBI Sequence Read Archive with the accession number of SRP129751. The acquired data were analyzed with methods as previously described (Shu et al., 2016).

3. Results and discussion

3.1. Simultaneous removals of V(V) and Cr(VI)

Gradual decreases in both V(V) and Cr(VI) were observed in three consecutive operating cycles in CS (Fig. 1a), indicating that simultaneous reductions of these two metal ions could be realized. In a typical cycle (72 h), V(V) removal efficiency was $97.0 \pm 1.0\%$ ($p < 0.05$), with nearly complete removal of Cr(VI) ($99.1 \pm 0.7\%$). The average removal rates were $15.8 \pm 0.3\,\mu M\,h^{-1}$ for V(V) and $20.3 \pm 0.4\,\mu M\,h^{-1}$ for Cr(VI) ($p < 0.05$), with pseudo first-order rate constants of 0.0335 h^{-1} and 0.0698 h^{-1}, correspondingly (Fig. 1b). These achievements showed remarkable advantage to results from pure culture. For instance, only 71.3% of V(V) and 91.2% of Cr(VI) were removed after 27-d operation by *Shewanella loihica* PV-4, with the same initial concentration and carbon source (Wang et al., 2017a). As metal ions could also be removed by adsorption (Saleh et al., 2017b), the obtained results were also compared with those from adsorption. For example, the maximum adsorption capacity of V(V) and Cr(VI) on Zr(IV)-impregnated collagen fiber was 1.92 mM g^{-1} and 0.53 mM g^{-1}, respectively (Liao et al., 2008). Our study provided more efficient and robust route to solving combined pollution in groundwater caused by these two toxic metal ions and broadened this proposed microbial reduction of contaminants for detoxification under anaerobic condition (Kotlarz et al., 2016; Tan et al., 2018; Li et al., 2018). It should be noted that results of this study were from a preliminary experiment in batch mode. Effects of groundwater chemistry and hydrodynamics were not considered yet. Further studies treating naturally V(V) and Cr(VI) co-contaminated groundwater under long term operation in
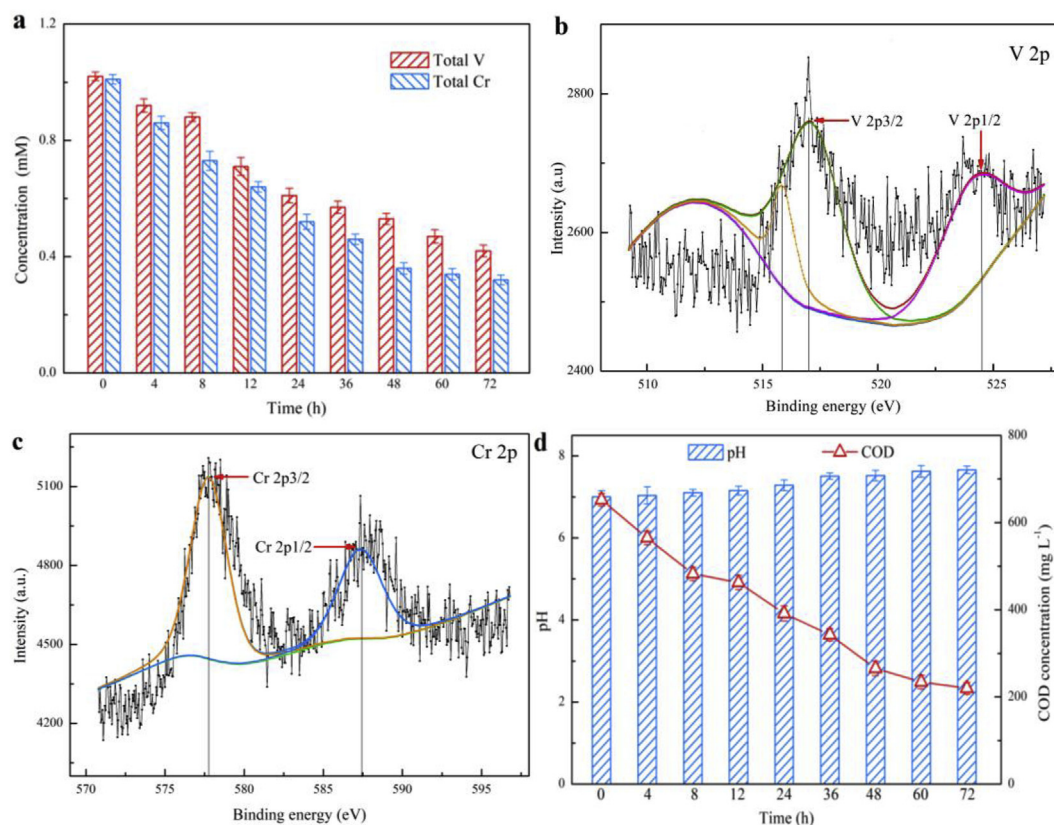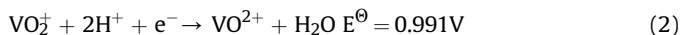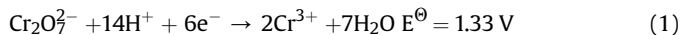

Fig. 2. Identification of reaction products in CS. (a) Changes of total V and total Cr in a typical cycle (72 h); (b) high resolution XPS spectrum of V 2p; (c) high resolution XPS spectrum of Cr 2p; (d) changes of pH and COD during 72-h operation.

continuous-flow mode were also required to further verify the gained results.

Concentrations of V(V) in SS-V and Cr(VI) in SS-Cr also decreased gradually with time (Fig. 1a), with reduction efficiency of $97.0 \pm 0.2\%$ and $86.1 \pm 1.2\%$ ($p < 0.05$), respectively. V(V) removal rate of $17.7 \pm 0.3 \, \mu M \, h^{-1}$ was obtained in SS-V, with pseudo first-order rate constant of $0.0461 \, h^{-1}$, while Cr(VI) removal rate was $11.9 \pm 0.4 \, \mu M \, h^{-1}$ in SS-Cr, with pseudo first-order rate constant of $0.0250 \, h^{-1}$ (Fig. 1b). These results were consistent with previous studies for bio-reductions of V(V) and Cr(VI) individually by mixed anaerobic culture (Zhang et al., 2015; Pradhan et al., 2017). Hardly any V(V) and Cr(VI) were removed in BS, demonstrating that V(V) and Cr(VI) removals in CS were biologically mediated and living microbes played crucial roles in detoxify these contaminants.

Interestingly, compared with SS-V and SS-Cr, Cr(VI) removal was accelerated, with lagged removal of V(V) in CS. These competitive and synergetic effects were also observed in co-contaminants removals by pure culture (Wang et al., 2017a). Firstly, Cr(VI) was given priority to gain electrons from the organics oxidation by microbes as it has higher redox potential than V(V) (Equation (1), (2)), thus its reduction process took priority in CS.

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O \quad E^{\Theta} = 1.33 \, V \quad (1)$$

$$VO_2^+ + 2H^+ + e^- \rightarrow VO^{2+} + H_2O \quad E^{\Theta} = 0.991 V \quad (2)$$

Secondly, Cr(VI) has a much higher biotoxicity than V(V) and microorganisms tend to reduce it first to less toxic and mobile
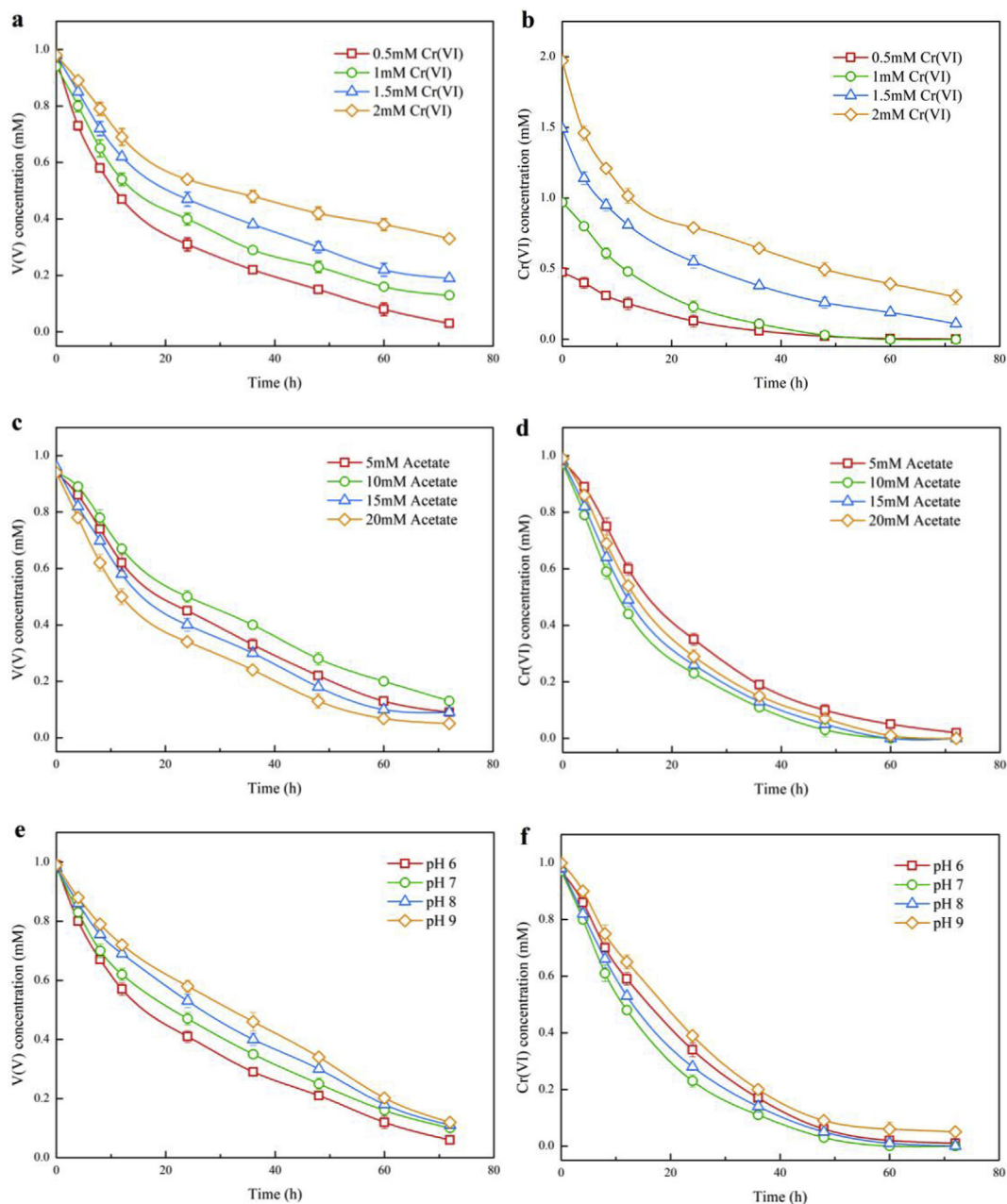

Fig. 3. Influences of operating factors on V(V) and Cr(VI) bio-reductions in CS. Removals of (a) V(V) and (b) Cr(VI) under different initial Cr(VI) concentrations with 1 mM V(V), 10 mM acetate and pH of 7; removals of (c) V(V) and (d) Cr(VI) under different initial acetate concentrations with 1 mM V(V), 1 mM Cr(VI) and pH of 7; removals of (e) V(V) and (f) Cr(VI) under different pH with initial 1 mM V(V), 1 mM Cr(VI) and 10 mM acetate.

Cr(III) by priority to improve its living condition (Singh et al., 2015). Under heavy metal ion conditions, proteins and proteases upregulated so as to aid in protein folding and promote refolding of polypeptides produced under stress (Bencheikh-Latmani et al., 2005). As Cr(VI) is more toxic than V(V), the proposed system possessed additional benefits with accelerated and enhanced Cr(VI) removal.

3.2. Examination of reduction products

Both total V and total Cr decreased progressively in the aqueous solution of CS in a typical cycle (72 h) (Fig. 2a), implying the insoluble characteristics of reduction products. Total Cr declined more significantly than total V, consistent with tendencies of V(V) and Cr(VI) in CS. After 72 h operation, removal efficiencies of total V and total Cr were $61.1 \pm 0.2\%$ and $68.3 \pm 0.3\%$ ($p < 0.05$), respectively. Meanwhile, obvious precipitates appeared during the operation of CS. They were collected and XPS survey indicated the peaks of vanadium and chromium, supporting decreases in total V and total Cr in the aqueous solution. High resolution spectra of V 2p were obtained and the detected sub-band located at 515.8 eV was identified as V(IV) (Fig. 2b) (Cai et al., 2017; Zhang et al., 2018). This result confirmed that V(V) was bio-reduced to V(IV). V(IV) was less toxic and could precipitate naturally in near-neutral conditions, with main forms of $VO(OH)_2$ and/or vanadyl phosphate $[CaV_2(PO_4)_2(OH)_4 \cdot 3H_2O]$ (Qiu et al., 2017). The generated V(IV) was easily re-oxidized when it was exposed to air during collections and tests, thus peaks corresponding to V(V) were also observed (Huang et al., 2015). Moreover, the Cr 2p3/2 - Cr 2p1/2 peak splitting value was observed to be 9.6 eV (Fig. 2c), good consistent with the literature value for Cr(III) (Li et al., 2008). The conditional solubility of Cr(III) under tested condition was negligible ($50–100 \mu g L^{-1}$) (Lai et al., 2016), thus the bio-generated Cr(III) could also precipitate spontaneously. Insolubilities of the reduction products of V(V) and Cr(VI) could realize removals of vanadium and chromium from groundwater, thus purifying V(V) and Cr(VI) co-contaminated environment efficiently.

Furthermore, slight increase in pH was observed in CS, due to the consumption of proton during bio-reductions of V(V) and Cr(VI) (Equation (1) and (2)) (Liu et al., 2017). COD decreased with time accordingly in CS (Fig. 2d), indicating the consumption of acetate during bio-reductions of V(V) and Cr(VI). This result was in accordance with results that most discovered V(V) and Cr(V) reducers were heterotrophic (Zhang et al., 2015; Pradhan et al., 2017), and acetate acted as electron donor and carbon source in this study. Two mechanisms were involved in metal ions bio-reduction, ie. microbes respired them directly via electron transfer, and/or they bound the reductases of other electron acceptors for detoxification (Yelton et al., 2013). Both pathways could occur in this study as mix anaerobic culture was employed, contributing to the enhancement of V(V) and Cr(VI) removals. The consumption of added COD was $432.6 \pm 4.9 mg L^{-1}$ in this process. When practical bioremediation was conducted, COD should be supplemented accurately, thus inhibition due to insufficient COD and secondary pollution caused by residual COD could be successfully prevented.

3.3. Influences of operating factors

Four gradients of initial Cr(VI) concentration (0.5 mM, 1 mM, 1.5 mM, 2 mM) were examined, with initial 1 mM V(V), 10 mM acetate and pH of 7. Though both V(V) and Cr(VI) were removed gradually within the 72-h operating period, V(V) removals were suppressed more obviously under higher initial Cr(VI) concentrations (Fig. 3a). V(V) reduction efficiencies decreased from $99.3 \pm 0.9\%$ at initial Cr(VI) concentration of 0.5 mM decreased to $67.0 \pm 1.2\%$ at initial Cr(VI) concentration of 2 mM ($p < 0.05$). V(V) removal rates were reduced from $13.1 \pm 0.1 \mu M h^{-1}$ to $9.0 \pm 0.2 \mu M h^{-1}$ accordingly. Regarding to Cr(VI), complete Cr(VI) removals happened with its initial concentrations below 1 mM, while Cr(VI) reduction efficiencies decreased to $85.0 \pm 0.6\%$ with its initial concentration of 2 mM (Fig. 3b). Nevertheless, Cr(VI) removal rates were enhanced from $6.5 \pm 0.2 \mu M h^{-1}$ to $23.3 \pm 0.1 \mu M h^{-1}$ with the increase in initial Cr(VI) concentration from 0.5 mM to 2 mM ($p < 0.05$). This suggested that the employed mixture anaerobic culture could realize higher Cr(VI) removals even under higher Cr(VI) concentration conditions.

As activities of dissimilatory metal reducing bacteria are affected by amount of electron donor and carbon source, experiments employing different initial acetate concentrations (5 mM, 10 mM, 15 mM, 20 mM) were conducted, with initial 1 mM V(V), 1 mM Cr(VI) and pH of 7. V(V) was removed more slowly than Cr(VI) under each tested condition (Fig. 3c and d). Notably, V(V) removal decreased first and then increased with the increase in initial acetate concentration, with the lowest V(V) reduction efficiency and removal rate at initial acetate concentration of 10 mM. Cr(VI) removals exhibited opposite tendencies, with maximum Cr(VI) removal efficiency and removal rate under 10 mM acetate condition. It was reported that approximate $330 mg L^{-1}$ COD was required for mix culture to reduce 1 mM V(V) (Zhang et al., 2015). Microbes tended to respire more V(V) with lower toxicity when carbon sources were limited while larger amount of carbon source could support higher microbial activities to respire more Cr(VI) with higher toxicity (Pradhan et al., 2017). When initial acetate concentration further increased, methanogenesis would compete with the dissimilatory metal reduction (Reul et al., 1999), which would reduce activities of dissimilatory metal reducing bacteria and resulted in the decrease of Cr(VI) removals.

Fig. 3e and f illustrated that V(V) and Cr(VI) removals varied under different pH (6, 7, 8, 9), with initial 1 mM V(V), 1 mM Cr(VI) and 10 mM acetate. Most V(V) and Cr(VI) could be removed, with faster Cr(VI) removal than V(V) under each tested pH, indicating that this proposed bioremediation of V(V) and Cr(VI) co-contaminated environment could function in a relatively wide pH range. pH played an important role in the toxicity of metals through affecting their speciation and it influenced contact between microorganisms and metal ions (Escudero et al., 2017; Bell et al., 2004; Li et al., 2016). At lower pH, dissolved V(V) and Cr(VI) are released into aqueous solution thus they can express their toxicities
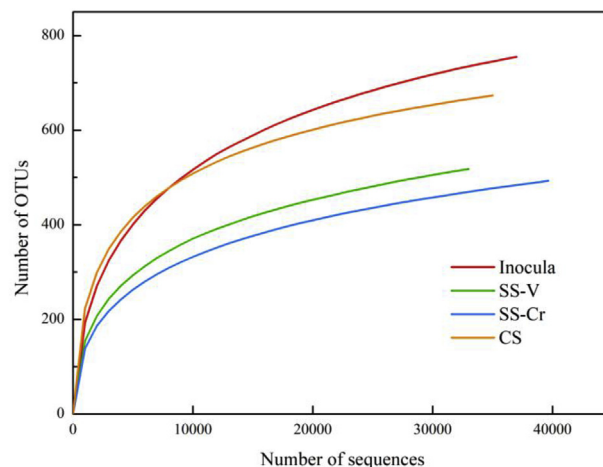

Fig. 4. Rarefaction curves based on sequencing of inoculated sludge and biomass in employed bioreactors. The OTUs were defined by 3% distances.

(Shaheen et al., 2016; Chen et al., 2015). Cr(VI) removal efficiencies and removal rates maximized under favorable condition (pH of 7), while adverse environment promoted V(V) removals, especially under acidic condition.

3.4. Dynamics of microbial community

Obvious changes took place in microbial richness and diversities. Microbial richness decreased, compared with the inocula,

Table 1
Alpha-diversity of microbial communities in inocula and bioreactors in this study.

	OUT number	Ace	Chao1	Shannon	Simpson	Coverage
Inocula	755	915	919	4.21	0.049	0.995
SS-V	518	653	680	4.21	0.034	0.996
SS-Cr	493	625	638	3.92	0.056	0.997
CS	673	774	776	4.84	0.018	0.996

as reflected by rarefaction curves drawn at a 3% distance (Fig. 4). Microbial growth and proliferation could be inhibited by the added V(V) and Cr(VI). SS-Cr possessed the lowest microbial richness, which could also be reflected by Ace and Chaol indexes (Table 1), as Cr(VI) is more toxic than V(V) (Zhang et al., 2017). However, microbial richness in CS was higher than that in SS-V and SS-Cr though lower than that in the inocula, implying antagonisms of added two metal ions (Hsieh et al., 2011). These effects were more obvious in aspects of microbial diversity, with the highest diversity of CS among the four samples (Table 1). Shannon and Simpson indexes suggested that the addition of Cr(VI) resulted in the decreased microbial diversity due to stronger toxicity, while microbial diversity increased slightly with the addition of V(V) as bacterial species were tolerant to V(V) up to 230 mg L^{-1} (Hao et al., 2016).

Evolution of microbial community was observed at phylum level (Fig. 5a). While *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* remained dominant with the addition of metal ions, the
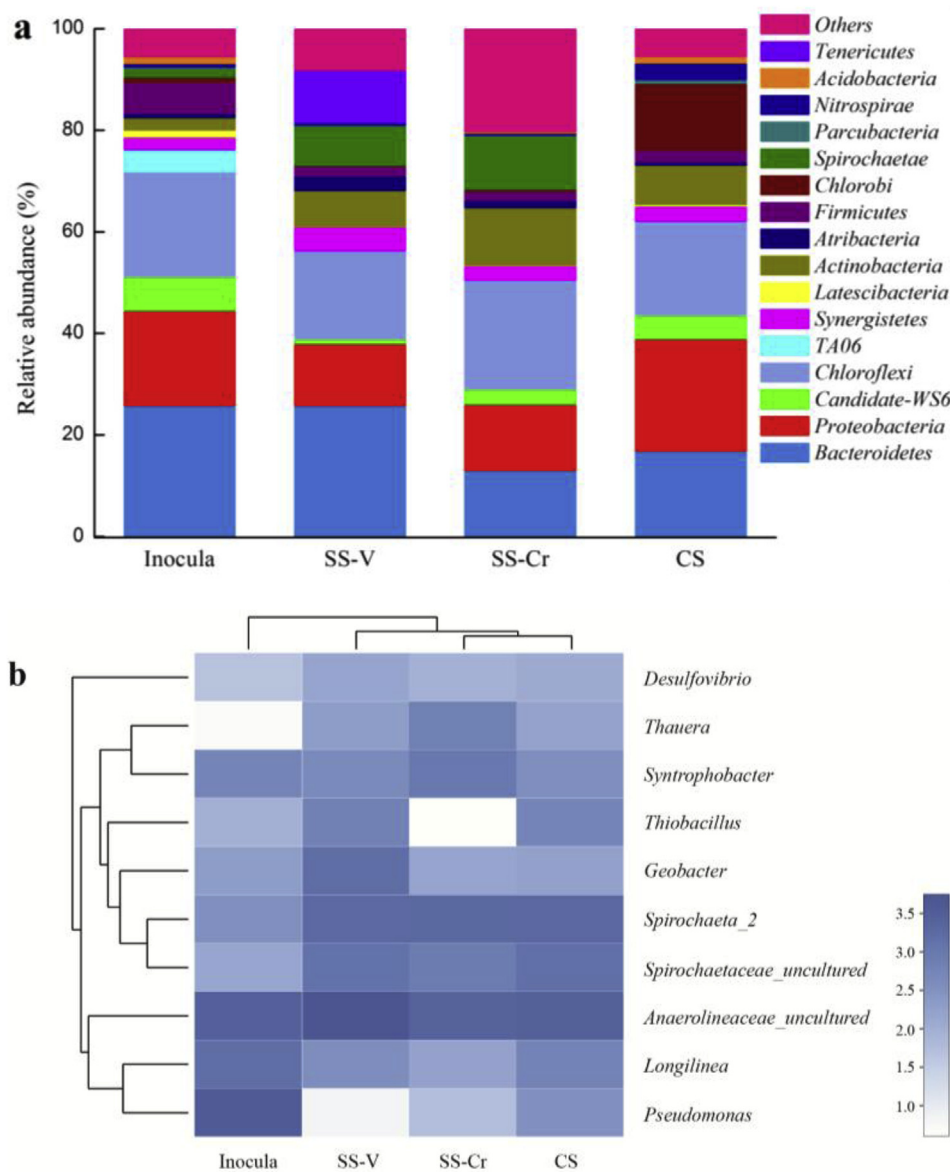

Fig. 5. Evolutions of microbial communities and functional species for inocula and employed bioreactors. (a) Microbial community compositions at phylum level; (b) functional genera involved in V(V) and Cr(VI) bio-reductions.

community structures with distinct survival conditions were noticeably different from that of the inocula. Specific phyla were accumulated significantly, such as *Synergistetes*, *Atribacteria* and *Tenericutes* in SS-V, consistent with previous studies (Liu et al., 2017). *Actinobacteria* and *Spirochaetae* appeared abundantly in SS-Cr, while *Chlorobi* and *Nitrospirae* possessed large portions in CS, which could participate in the detoxification of added metal ions.

Functional species possibly involved in bio-reductions of V(V) and Cr(VI) were found at genus level (Fig. 5b). A large number of *Geobacter* appeared newly in SS-V, which was previously reported to reduce V(V) (Ortiz-Bernad et al., 2004). *Anaerolineaceae* was accumulated significantly in SS-V (17%) and it was reported to contribute to selenate reduction with selenate reductases in the Rifle site (Fakra et al., 2018). *Thiobacillus*, whose abundance increased to 1.9% in SS-V was reported to reduced nitrate and nitrite through extracellular electron transfer (Pous et al., 2014). Enrichment of *Thauera* genus was obvious in SS-Cr and its role in Cr(VI) detoxification and immobilization had been revealed (Miao et al., 2015). *Syntrophobacter* also boosted in SS-Cr and it was able to use nitrite, nitrate and/or sulfate as the electron acceptor (Ramos et al., 2016; Worm et al., 2014). These mentioned genera above were also detected in CS, except *Geobacter*, which might be responsible for V(V) and Cr(VI) reductions directly or indirectly in CS. Particularly, proportions of *Spirochaeta* and *Spirochaetaceae* were the largest in CS among all samples and they could also account for V(V) and Cr(VI) removals with their abilities of reducing sulfate and Fe(III) (Kümmel et al., 2015; Baek et al., 2016).

4. Conclusions

Simultaneous bio-reductions of V(V) and Cr(VI) by mixed anaerobic culture were realized with high efficiency in this study. Removal efficiencies of V(V) and Cr(VI) after 72-h operation were $97.0 \pm 1.0\%$ and $99.1 \pm 0.7\%$, respectively. Interactions between V(V) and Cr(VI) were revealed, with promoted Cr(VI) bio-reduction and suppressed V(V) removal. V(IV) and Cr(III) were the main reduction products, which could precipitate under near-neutral groundwater. Influences of initial Cr(VI) and acetate concentrations and pH were also investigated. Microbial communities evolved and specific species such as *Anaerolineaceae*, *Spirochaeta* and *Spirochaetaceae* were enriched, which could be involved in bio-reductions of V(V) and Cr(VI). This study reveals the relationship between V(V) and Cr(VI) in aquifer during their bio-reductions and is helpful to develop novel technology for remediation of V(V) and Cr(VI) co-contaminated groundwater.

Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.06.080.

References

Altıntıg, E., Altundag, H., Tuzen, M., Sarı, A., 2017. Effective removal of methylene blue from aqueous solutions using magnetic loaded activated carbon as novel adsorbent. Chem. Eng. Res. Des. 122, 151–163.

Baek, G., Kim, J., Shin, S.G., Lee, C., 2016. Bioaugmentation of anaerobic sludge digestion with iron-reducing bacteria: process and microbial responses to variations in hydraulic retention time. Appl. Microbiol. Biotechnol. 100, 927–937.

Bell, J.M., Philp, J.C., Kuyukina, M.S., Ivshina, I.B., Dunbar, S.A., Cunningham, C.J., Anderson, P., 2004. Methods evaluating vanadium tolerance in bacteria isolated from crude oil contaminated land. J. Microbiol. Meth. 58 (1), 87–100.

Bencheikh-Latmani, R., Williams, S.M., Haucke, L., Criddle, C.S., Wu, L., Zhou, J., Tebo, B.M., 2005. Global transcriptional profiling of *Shewanella oneidensis* MR-1 during Cr(VI) and U(VI) reduction. Appl. Environ. Microbiol. 71 (11), 7453–7460.

Cai, R.Q., Zhang, B.G., Shi, J.X., Li, M., He, Z., 2017. Rapid photocatalytic decolorization of methyl orange under visible light using VS4/carbon powder nanocomposites. ACS Sustain. Chem. Eng. 5, 7690–7699.

Cao, X.L., Diao, M.H., Zhang, B.G., Liu, H., Wang, S., Yang, M., 2017. Spatial distribution of vanadium and microbial community responses in surface soil of Panzhihua mining and smelting area, China. Chemosphere 183, 9–17.

Chebeir, M., Chen, G., Liu, H., 2016. Emerging investigators series: frontier review: occurrence and speciation of chromium in drinking water distribution systems. Environ. Sci.: Water Res. Technol 2, 906–914.

Chen, G., Liu, H., 2017. Understanding the reduction kinetics of aqueous vanadium (V) and transformation products using rotating ring-disk electrodes. Environ. Sci. Technol. 51 (20), 11643–11651.

Chen, T., Zhou, Z., Xu, S., Wang, H., Lu, W., 2015. Adsorption behavior comparison of trivalent and hexavalent chromium on biochar derived from municipal sludge. Bioresour. Technol. 190, 388–394.

Cho, K.C., Lee, D.G., Roh, H., Fuller, M.E., Hatzinger, P.B., Chu, K.H., 2013. Application of 13 C-stable isotope probing to identify RDX-degrading microorganisms in groundwater. Environ. Pollut. 178, 350–360.

Deng, J., Huang, K.H., An, L., Hu, C.H., Ju, S.Q., Xiao, N., 2014. Cloud point extraction and simultaneous spectrophotometric determination of V(V), Co(II) and Cu(II) ions in water samples by 5-Br-PADAP using partial least squares regression. J. Radioanal. Nucl. Chem. 300, 835–842.

Escudero, C., Fiol, N., Villaescusa, I., Bollinger, J.C., 2017. Effect of chromium speciation on its sorption mechanism onto grape stalks entrapped into alginate beads. Arab. J. Chem. 10, S1293–S1302.

Fang, D., Zhang, X., Dong, M., Xue, X., 2017. A novel method to remove chromium, vanadium and ammonium from vanadium industrial wastewater using a byproduct of magnesium-based wet flue gas desulfurization. J. Hazard Mater. 336, 8–20.

Fakra, S.C., Luef, B., Castelle, C.J., Mullin, S.W., Williams, K.H., Marcus, M.A., Schichnes, D., Banfield, J.F., 2018. Correlative cryogenic spectro-microscopy to investigate Selenium bioreduction products. Environ. Sci. Technol. 52, 503–512.

Gong, Y.F., Werth, C.J., He, Y.X., Su, Y.M., Zhang, Y.L., Zhou, X.F., 2018. Intracellular versus extracellular accumulation of Hexavalent chromium reduction products by *Geobacter sulfurreducens* PCA. Environ. Pollut. 240, 485–492.

Hao, L.T., Zhang, B.G., Tian, C.X., Liu, Y., Shi, C.H., Cheng, M., Feng, C.P., 2015. Enhanced microbial reduction of vanadium (V) in groundwater with bioelectricity from microbial fuel cells. J. Power Sources 287, 43–49.

Hao, L.T., Zhang, B.G., Cheng, M., Feng, C.P., 2016. Effects of various organic carbon sources on simultaneous V (V) reduction and bioelectricity generation in single chamber microbial fuel cells. Bioresour. Technol. 201, 105–110.

Hsieh, B.T., Chang, C.Y., Chang, Y.C., Cheng, K.Y., 2011. Relationship between the level of essential metal elements in human hair and coronary heart disease. J. Radioanal. Nucl. Chem. 290 (1), 165–169.

Huang, D.D., Wang, G.C., Shi, Z.M., Li, Z.H., Kang, F., Liu, F., 2017. Removal of hexavalent chromium in natural groundwater using activated carbon and cast iron combined system. J. Clean. Prod. 165, 667–676.

Huang, J.H., Huang, F., Evans, L., Glasauer, S., 2015. Vanadium: global (bio) geochemistry. Chem. Geol. 417, 68–89.

Jiang, Y.F., Zhang, B.G., He, C., Shi, J.X., Borthwick, A.G.L., Huang, X.Y., 2018. Synchronous microbial vanadium (V) reduction and denitrification in groundwater using hydrogen as the sole electron donor. Water Res. 141, 289–296.

Kotlarz, N., Upadhyaya, G., Togn, P., Raskin, L., 2016. Evaluation of electron donors for biological perchlorate removal highlights the importance of diverse perchlorate-reducing populations. Environ. Sci.: Water Res. Technol 2, 1049–1063.

Kümmel, S., Herbst, F.A., Bahr, A., Duarte, M., Pieper, D.H., Jehmlich, N., Seifert, J., von Bergen, M., Bombach, P., Richnow, H.H., 2015. Anaerobic naphthalene degradation by sulfate-reducing Desulfobacteraceae from various anoxic aquifers. FEMS Microbiol. Ecol. 91 fiv006.

Lai, C.Y., Zhong, L., Zhang, Y., Chen, J.X., Wen, L.L., Shi, L.D., Sun, Y.P., Ma, F., Rittmann, B., Zhou, C., Tang, Y., Zheng, P., Zhao, H.P., 2016. Bioreduction of chromate in a methane-based membrane biofilm reactor. Environ. Sci. Technol. 50 (11), 5832–5839.

Li, B., Pan, D., Zheng, J., Cheng, Y., Ma, X., Huang, F., Lin, Z., 2008. Microscopic investigations of the Cr (VI) uptake mechanism of living Ochrobactrum anthropi. Langmuir 24 (17), 9630–9635.

Li, F., Xia, Q., Gao, Y., Cheng, Q., Ding, L., Yang, B., Tian, Q., Ma, C., Sand, W., Liu, Y., 2018. Anaerobic biodegradation and decolorization of a refractory acid dye by a forward osmosis membrane bioreactor. Environ. Sci.: Water Res. Technol 4, 272–280.

Li, J., Jin, R., Liu, G., Tian, T., Wang, J., Zhou, J., 2016. Simultaneous removal of as carbon source and biofilm carriers. Bioresour. Technol. 207, 308–314.

Li, Y., Low, G.K.-C., Scott, J.A., Amal, R., 2007. Microbial reduction of hexavalent chromium by landfill leachate. J. Hazard Mater. 142, 153—159.

Liao, X.P., Tang, W., Zhou, R.Q., Shi, B., 2008. Adsorption of metal anions of vanadium(V) and chromium(VI) on Zr(IV)-impregnated collagen fiber. Adsorption 14, 55—64.

Liu, H., Zhang, B.G., Xing, Y., Hao, L.T., 2016. Behavior of dissolved organic carbon sources on the microbial reduction and precipitation of vanadium (V) in groundwater. RSC Adv. 6, 97253—97258.

Liu, H., Zhang, B.G., Yuan, H.Y., Cheng, Y., Wang, S., He, Z., 2017. Microbial reduction of vanadium (V) in groundwater: interactions with coexisting common electron acceptors and analysis of microbial community. Environ. Pollut. 231, 1362—1369.

Liu, J., He, X.X., Lin, X.R., Chen, W.C., Zhou, Q.X., Shu, W.S., Huang, L.N., 2015. Ecological effects of combined pollution associated with e-waste recycling on the composition and diversity of soil microbial communities. Environ. Sci. Technol. 49 (11), 6438—6447.

Miao, Y., Liao, R.H., Zhang, X.X., Wang, Y., Wang, Z., Shi, P., Liu, B., Li, A., 2015. Metagenomic insights into Cr(VI) effect on microbial communities and functional genes of an expanded granular sludge bed reactor treating high-nitrate wastewater. Water Res. 76, 43—52.

Mitchell, E., Frisbie, S., Sarkar, B., 2011. Exposure to multiple metals from groundwater-a global crisis: geology, climate change, health effects, testing, and mitigation. Metall 3, 874—908.

Mochizuki, M., Ueda, F., Sasaki, S., Hondo, R., 1999. Vanadium contamination and the relation between vanadium and other elements in wild birds. Environ. Pollut. 106, 249—251.

Narayani, M., Shetty, K.V., 2013. Chromium-resistant bacteria and their environmental condition for hexavalent chromium removal: a review. Crit. Rev. Environ. Sci. Technol. 43 (9), 955—1009.

Němeček, J., Pokorný, P., Lhotský, O., Knytl, V., Najmanová, P., Steinová, J., Černík, M., Filipová, A., Filip, J., Cajthaml, T., 2016. Combined nano-biotechnology for in-situ remediation of mixed contamination of groundwater by hexavalent chromium and chlorinated solvents. Sci. Total Environ. 563—564, 822—834.

Ortiz-Bernad, I., Anderson, R.T., Vrionis, H.A., Lovley, D.R., 2004. Vanadium respiration by Geobacter metallireducens: novel strategy for in situ removal of vanadium from groundwater. Appl. Environ. Microbiol. 70, 3091—3095.

Pous, N., Koch, C., Colprim, J., Puig, S., Harnisch, F., 2014. Extracellular electron transfer of biocathodes: revealing the potentials for nitrate and nitrite reduction of denitrifying microbiomes dominated by Thiobacillus sp. Electrochem. Commun. 49, 93—97.

Pradhan, D., Sukla, L.B., Sawyer, M., Rahman, P.K., 2017. Recent bioreduction of hexavalent chromium in wastewater treatment: a review. J. Ind. Eng. Chem. 55, 1—20.

Qiu, R., Zhang, B.G., Li, J.X., Lv, Q., Wang, S., Gu, Q., 2017. Enhanced vanadium (V) reduction and bioelectricity generation in microbial fuel cells with biocathode. J. Power Sources 359, 379—383.

Ramos, C., Suárez-Ojeda, M.E., Carrera, J., 2016. Denitritation in an anoxic granular reactor using phenol as sole organic carbon source. Biochem. Eng. J. 288, 289—297.

Rasheed, M.A., Radha, B.A., Rao, P.S., Lakshmi, M., Chennaiah, J.B., Dayal, A.M., 2012. Evaluation of potable groundwater quality in some villages of Adilabad in Andhra Pradesh, India. J. Environ. Biol. 33 (4), 689.

Reijonen, I., Metzler, M., Hartikainen, H., 2016. Impact of soil pH and organic matter on the chemical bioavailability of vanadium species: the underlying basis for risk assessment. Environ. Pollut. 210, 371—379.

Reul, B.A., Amin, S.S., Buchet, J.P., Ongemba, L.N., Crans, D.C., Brichard, S.M., 1999. Effects of vanadium complexes with organic ligands on glucose metabolism: a comparison study in diabetic rats. Br. J. Pharmacol. 126, 467—477.

Saleh, T.A., Sarı, A., Tuzen, M., 2017a. Effective adsorption of antimony(III) from aqueous solutions by polyamide-graphene composite as a novel adsorbent. Chem. Eng. J. 307, 230—238.

Saleh, T.A., Tuzen, M., Sarı, A., 2017b. Magnetic activated carbon loaded with tungsten oxide nanoparticles for aluminum removal from waters. J. Environ. Chem. Eng 5, 2853—2860.

Saleh, T.A., Tuzen, M., Sarı, A., 2018. Polyamide magnetic palygorskite for the simultaneous removal of Hg(II) and methyl mercury; with factorial design analysis. J. Environ. Manag. 211, 323—333.

Shaheen, S.M., Rinklebe, J., Frohne, T., White, J.R., DeLaune, R.D., 2014a. Biogeochemical factors governing cobalt, nickel, selenium, and vanadium dynamics in periodically flooded Egyptian north Nile Delta rice soils. Soil Sci. Soc. Am. J. 78, 1065—1078.

Shaheen, S.M., Rinklebe, J., Rupp, H., Meissner, R., 2014b. Lysimeter trials to assess the impact of different flood-dry-cycles on the dynamics of pore water concentrations of As, Cr, Mo and V in a contaminated floodplain soil. Geoderma 228—229, 5—13.

Shaheen, S.M., Rinklebe, J., Frohne, T., White, J.R., DeLaune, R.D., 2016. Redox effects on release kinetics of arsenic, cadmium, cobalt, and vanadium in Wax Lake Deltaic freshwater marsh soils. Chemosphere 150, 740—748.

Shu, D., He, Y., Yue, H., Wang, Q., 2016. Metagenomic and quantitative insights into microbial communities and functional genes of nitrogen and iron cycling in twelve wastewater treatment systems. Chem. Eng. J. 290, 21—30.

Singh, R., Dong, H., Liu, D., Zhao, L., Marts, A.R., Farquhar, E., Tierney, D.L., Almquist, C.B., Briggs, B.R., 2015. Reduction of hexavalent chromium by the thermophilic methanogen Methanothermobacter thermautotrophicus. Geochem. Cosmochim. Acta 148, 442—456.

Tan, L., Nancharaiah, Y., Hullebusch, E., Lens, P., 2018. Effect of elevated nitrate and sulfate concentrations on selenate removal by mesophilic anaerobic granular sludge bed reactors. Environ. Sci.: Water Res. Technol 4, 303—314.

Wang, G.Y., Zhang, B.G., Li, S., Yang, M., Yin, C., 2017a. Simultaneous microbial reduction of vanadium (V) and chromium (VI) by Shewanella loihica PV-4. Bioresour. Technol. 227, 353—358.

Wang, M., Chen, B., Huang, S., Wang, X., Liu, B., Ge, Q., Xie, S., 2017b. A novel technology for vanadium and chromium recovery from V-Cr-bearing reducing slag. Hydrometallurgy 171, 116—122.

Wang, W., Zhang, B.G., Liu, Q., Du, P., Liu, W., He, Z., 2018. Biosynthesis of palladium nanoparticles using Shewanella loihica PV-4 for excellent catalytic reduction of chromium (VI). Environ. Sci Nano 5, 730—739.

Worm, P., Koehorst, J.J., Visser, M., Sedano-Núñez, V.T., Schaap, P.J., Plugge, C.M., Sousa, D.Z., Stams, A.J., 2014. A genomic view on syntrophic versus non-syntrophic lifestyle in anaerobic fatty acid degrading communities. Biochim. Biophys. Acta 1837, 2004—2016.

Yang, J., Wang, M., Jia, Y., Gou, M., Zeyer, J., 2017. Toxicity of vanadium in soil on soybean at different growth stages. Environ. Pollut. 231, 48—58.

Yelton, A.P., Williams, K.H., Fournelle, J., Wrighton, K.C., Handley, K.M., Banfield, J.F., 2013. Vanadate and acetate biostimulation of contaminated sediments decreases diversity, selects for specific taxa, and decreases aqueous V^{5+} concentration. Environ. Sci. Technol. 47 (12), 6500—6509.

Yurkow, E.J., Hong, J.H., Min, S., Wang, S., 2002. Photochemical reduction of hexavalent chromium in glycerol-containing solutions. Environ. Pollut. 117, 1—3.

Zhang, B.G., Feng, C.P., Ni, J.R., Zhang, J., Huang, W., 2012. Simultaneous reduction of vanadium (V) and chromium (VI) with enhanced energy recovery based on microbial fuel cell technology. J. Power Sources 204, 34—39.

Zhang, B.G., Hao, L.T., Tian, C.X., Yuan, S., Feng, C.P., Ni, J.R., Borthwick, A.G., 2015. Microbial reduction and precipitation of vanadium (V) in groundwater by immobilized mixed anaerobic culture. Bioresour. Technol. 192, 410—417.

Zhang, B., Zhao, H., Zhou, S., Shi, C., Wang, C., Ni, J., 2009. A novel UASB-MFC-BAF integrated system for high strength molasses wastewater treatment and bioelectricity generation. Bioresour. Technol. 100 (23), 5687—5693.

Zhang, B.G., Zou, S.Q., Cai, R.Q., Li, M., He, Z., 2018. Highly-efficient photocatalytic disinfection of Escherichia coli under visible light using carbon supported Vanadium Tetrasulfide nanocomposites. Appl. Catal. B Environ. 224, 383—393.

Zhang, J., Chen, L., Yin, H., Jin, S., Liu, F., Chen, H., 2017. Mechanism study of humic acid functional groups for Cr(VI) retention: two-dimensional FTIR and ^{13}C CP/MAS NMR correlation spectroscopic analysis. Environ. Pollut. 225, 86—92.

Zhang, J., Dong, H., Zhao, L., McCarrick, R., Agrawal, A., 2014. Microbial reduction and precipitation of vanadium by mesophilic and thermophilic methanogens. Chem. Geol. 370, 29—39.

Zhong, L., Lai, C.Y., Shi, L.D., Wang, K.D., Dai, Y.J., Liu, Y.W., Ma, F., Rittmann, B.E., Zheng, P., Zhao, H.P., 2017. Nitrate effects on chromate reduction in a methane-based biofilm. Water Res. 115, 130—137.