Microbial reduction and precipitation of vanadium (V) in groundwater by immobilized mixed anaerobic culture

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HIGHLIGHTS

- Vanadium (V) is effectively reduced by immobilized mixed anaerobic sludge.
- V(IV) is the main reduction product and it precipitates instantly from groundwater.
- Initial V(V), COD concentrations, pH and conductivity affect the performance.
- High-throughput pyrosequencing analysis indicates the decreased diversity.
- New functional species as Lactococcus, Enterobacter and Spirochaeta are found.

ABSTRACT

Vanadium is an important contaminant impacted by natural and industrial activities. Vanadium (V) reduction efficiency as high as 87.0% was achieved by employing immobilized mixed anaerobic sludge as inoculated seed within 12 h operation, while V(IV) was the main reduction product which precipitated instantly. Increasing initial V(V) concentration resulted in the decrease of V(V) removal efficiency, while this index increased first and then decreased with the increase of initial COD concentration, pH and conductivity. High-throughput 16S rRNA gene pyrosequencing analysis indicated the decreased microbial diversity. V(V) reduction was realized through dissimilatory reduction process by significantly enhanced Lactococcus and Enterobacter with oxidation of lactic and acetic acids from fermentative microorganisms such as the enriched Paludibacter and the newly appeared Acetobacterium, Oscillibacter. This study is helpful to detect new functional species for V(V) reduction and constitutes a step ahead in developing in situ bioremediations of vanadium contamination.

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1. Introduction

Vanadium widely exists in the Earth’s crust (Rehder, 1991). It is also a valuable metal as well as catalyst extensively used in modern technologies including metallurgy, petroleum refining and production of phthalic anhydrides (Zhang et al., 2014). Thus vanadium contamination in groundwater can result from either natural or industrial sources (Ortiz-Bernad et al., 2004; Naeem et al., 2007). It is moderately toxic and becomes toxic to animal cells at concentrations greater than 1–10 μg/L (Yelton et al., 2013). The toxicity of vanadium increases with its valence state and solubility. Vanadium (V) (V(V)) is considered as the most toxic and mobile form while V(IV) is less toxic and insoluble at neutral pH (Wang and Ren, 2014). Consequently, reduction of V(V) to V(IV) is considered as a promising remediation method to remove vanadium from contaminated groundwater (Ortiz-Bernad et al., 2004).

Since 1970s, various microorganisms classified in bacterial, eukaryotic or archaeal domain have been reported to possess the ability of reducing V(V) to V(IV). Metal reducing bacteria Geobacter metallireducens and Shewanella oneidensis are capable of growth with V(V) as the sole electron acceptor (Carpentier et al., 2003, 2005; Ortiz-Bernad et al., 2004). Several Pseudomonas strains have also been reported to be capable of reducing V(V) to...
lower oxidation states (V(III) and V(IV)) (Lyalikova and Yurkova, 1992). Both mesophilic and thermophilic methanogens are also able to reduce V(V) (Zhang et al., 2014). These studies demonstrate the possibility of using microbial approach to remediate toxic vanadium contaminated environment. However, most of these studies are carried out by testing the ability of the V(V) reduction in pure cultures, instead of isolating functional microbes from common mixed cultures (Yelton et al., 2013). A single microbial strain only functions in a narrow range of substrates and operating conditions with relatively lower efficiency, thus more V(V) reducing microbes should be discovered and coupled with others to promote V(V) contamination remediation. Using mixed cultures can handle the complex conditions with the higher microbial diversity, great adaption and self-evolution abilities (Liang et al., 2014). Mixed cultures are easily available and feed sterilization is not required when compared to pure cultures, thus they are more suitable for actual remediation applications (Veeravalli et al., 2014). Mixed microbial consortia have been applied to environmental protection under aerobic or anaerobic conditions for more than a century (Agler et al., 2011). However, limited studies using mixed anaerobic cultures for V(V) reduction have been reported (Yelton et al., 2013). To the authors’ best knowledge, there have been papers that focus on biological V(V) reduction using anaerobic sludge, which is the most common mixed culture in wastewater treatment processes. Moreover, anaerobic sludge possesses a variety of microorganisms, which is propitious to the discovery of new functional V(V) reducing microbes.

In the present study, the feasibility of microbial reduction and precipitation of V(V) with common anaerobic sludge as inoculums seed was investigated. Factors affecting the system performance including initial V(V) as well as chemical oxygen demand (COD) concentration, pH and conductivity were examined. The involved microbes were also analyzed and new functional spieces were detected. Reduction products were also studied meanwhile. This study provided new microbial sources and results had promising application prospects for remediation of vanadium contaminated environment.

2. Methods

2.1. Bioreactors construction and inoculated culture

Ten plexiglass bioreactors designed in sealed cuboid shape with total volume of 480 mL were employed. Two pieces of conductive carbon fiber felt with dimensions of 40 × 40 × 10 mm were inserted into the bioreactors for microbes and deposits attachment. Each reactor was filled with 400 mL solution containing the following components (per L): 0.75 g of CaH2O2; 4.97 g of NaH2PO4·2H2O; 2.75 g of Na2HPO4·12H2O; 0.31 g of NH4Cl; 0.13 g of KCl; 1.25 mL of vitamin solution; and 12.5 mL of trace mineral element solution (Lovley and Phillips, 1988). Conductivities were adjusted to 12 mS/cm with the addition of NaCl. V(V) was added into above solution in the form of NaVO3 with the given concentration. The initial solution pH was maintained about 7 with the addition of phosphate buffer solution (50 mM). Two of the reactors were inoculated with 50 mL anaerobic sludge obtained from an up-flow anaerobic sludge blanket (UASB) reactor treating high strength sulfate wastewater as the formal experimental apparatus (Bioreactor). The left were control sets and were divided into four groups equally. One group was not inoculated (Control 1) and another was inoculated with 50 mL anaerobic sludge with high temperature sterilization (Control 2). Both third and fourth groups were also inoculated with 50 mL anaerobic sludge without sterilization but glucose was omitted from the substrate in Control 3 and carbon fiber felt was removed to form free suspended bacterial solution in Control 4.

2.2. Operation of the bioreactors

The two inoculated bioreactors were domesticated for 3 months before the formal experiments. The aqueous solution with V(V) of 75 mg/L was refreshed each day during this period. After accumulation, the feasibility of microbial V(V) reduction was evaluated, compared with control sets. The 12 h fed-batch mode was chosen as most V(V) was reduced in the bioreactors within that time. The responding reduced products were also monitored. After that, factors affecting the bioreactors performance were examined separately, including initial V(V) concentrations (50 mg/L, 75 mg/L, 150 mg/L, 300 mg/L), initial COD concentrations (200 mg/L, 800 mg/L, 1200 mg/L, 1600 mg/L), pH (5.4, 6.2, 7.0, 7.8) and conductivities (10 mS/cm, 12 mS/cm, 15 mS/cm, 19 mS/cm). When one factor was studied, others were fixed at chosen values. pH experiments were conducted in corresponding phosphate buffered saline with the same amount of substance concentration (50 mM). Conductivities were adjusted by adding different amounts of NaCl. Then PCR and high-throughput sequencing analysis were performed to obtain the strains information and their effects on V(V) reduction after another 3 months accumulation. The two bioreactors as well as controls in each group were operated under identical conditions and their average results were recorded. All the experiments were conducted at room temperature (22 ± 2 °C).

2.3. Analytical methods and microbiological analysis

Spectrophotometric methods were chosen to measure the reduction of V(V) and generation of V(IV) (Safavi et al., 2000; Ensafi et al., 1999). Total vanadium was determined by ICP-MS (Thermo Fisher X series, Germany). COD was measured by fast air-tight catalytic decomposition method. pH was measured using a
pH-201 meter (Hanna, Italy). Electrolyte conductivity was monitored by a conductivity meter (DDS-11A, Shanghai Lei Yun test equipment Manufacturing Co., Ltd., Shanghai, China).

The surface morphology of the carbon fiber felt was examined by scanning electron microscopy (SEM) (Quanta, FEI Co., Hillsboro, OR, USA) after the whole experiment. The deposits on the surface of carbon fiber felt were determined by Energy dispersive X-ray (EDX) and X-ray photoelectron spectroscopy (XPS) (Axis Ultra, Kratos Analytical Ltd., Manchester, UK).

Molecular biology analysis was carried out to acquire characteristics of microbial population. Ultrasonic was employed to collect the bacteria attached to the surface of carbon fiber felt in the bioreactor and the bacteria in the inoculated sludge at the same time. Total genomic DNA was extracted from both samples using FastDNA SPIN Kit for Soil (Qiagen, CA, The USA) according to the manufacturer’s instructions. Then the above DNA was pooled and amplified by PCR (GeneAmp 9700, ABI, The USA). After being purified and quantified, a mixture of amplicons was used for high-throughput 16S rRNA gene pyrosequencing on MiSeq (Illumina, The USA). Raw pyrosequencing data that obtained from this study were deposited to the NCBI Sequence Read Archive Database.

3. Results and discussion

3.1. Microbial performance of V(V) reduction

When the acclimated mixed culture was inoculated into freshwater medium containing 75 mg/L V(V) (1.47 mM), obvious V(V) removal was obtained (Fig. 1), demonstrating that the mixture culture functioned well for V(V) reduction. At the end of the operating cycle (12 h), 87.0% of V(V) was removed. This was more effective than results from the reported pure cultures. For example, 6 d was required for *G. metallireducens* to completely reduce 1 mM V(V) (Ortiz-Bernad et al., 2004), while 2 mM of V(V) was reduced by mesophilic and thermophilic methanogens after 30 d (Zhang et al., 2014). Rare V(V) reduction was observed in Control 1 when the freshwater medium was not inoculated with live cells, while slight V(V) reduction happened in Control 2 when the mixed anaerobic culture was heat killed before incubation (Fig. 1), probably due to the adsorption of the sludge flocs and the function of residual live cells. It was reported that V(V) bioreduction occurred in one of two ways for pure cultures, ie. microbial V(V) respiration via electron transfer or V(V) detoxification as the result of vanadium binding to reductases of other electron acceptors and precipitation of an insoluble V(IV) phase (Yelton et al., 2013). These two effects might both happen with the mixed anaerobic culture, thus accelerating the performance of microbial V(V) reduction in present research. Moreover, the advantages of the mixed anaerobic sludge were also included (i) presence of high microbial diversity offering increased adaptation capacity, (ii) possibility of mixed substrates co-fermentation, and (iii) higher capacity for continuous processing (Singla et al., 2014). The mixed anaerobic sludge presented an opportunity for higher microbial V(V) reduction efficiency. Meanwhile, the addition of organics (glucose) in the bioreactor was effective for enhancement of microbial V(V) reduction, compared with Control 3 which had only endogenous respirations (Fig. 1) and previous study (Yelton et al., 2013). Biostimulation by organics amendment could be conductive to management of vanadium contamination in groundwater. The
immobilized anaerobic biofilm in the bioreactor also performed better regard to microbial V(V) reduction compared with Control 4 with free suspended culture as well as other research (Yelton et al., 2013). Carbon fiber felt is a kind of satisfactory conductive materials and can facilitate bacterial extracellular electron transfer, which had been demonstrated in electrochemical system studies as it was widely employed as anode electrode in microbial fuel cells (MFCs) for electricigens attachment and electron delivery (Zhang et al., 2012). Immobilized anaerobic biofilm was effective in the aspect of microbial V(V) reduction area, implying an advanced strategy for microbial performance enhancement. In another aspect, the removal rate of V(V) with time fitted a pseudo-first-order reaction basically in the 12 h operation. The kinetic equations and parameters were shown in Table 1, from which it could be seen that the kinetic constant of V(V) removal in the bioreactor was greater than that in the controls, again suggesting that V(V) was reduced more quickly in the proposed system.

V(V) was mainly transformed into V(IV) as the concentration of V(IV) increased accordingly in Fig. 1, with other species of vanadium undetected. The color of the medium changed from yellow-brown to blue in the bioreactor, attributable to the presence of V(IV) in the form of the vanadyl ion (Wang and Ren, 2014). There had been two mechanisms of microbial V(V) reduction to V(IV) as intracellular and membrane-associated processes (Zhang et al., 2014). Both of these two pathways could function simultaneously in the bioreactor and thus the efficiencies were improved. Additionally, green precipitate was also found accumulated on the surface of carbon fiber by visual observation and SEM analysis. EDX analysis indicated that the precipitate was mainly comprised of vanadium and phosphorous, suggesting that it could be a vanadyl phosphate, such as the green mineral sincosite [CaV2(PO4)3(OH)6H2O], which had also been reported before (Ortiz-Bernad et al., 2004; Zhang et al., 2014). Two obvious broad V 2p peaks appeared for these precipitates during XPS test, i.e. peaks located at 516.8 eV and 524.5 eV, corresponding to V 2p3/2 and V 2p1/2, respectively. The V 2p3/2−V 2p1/2 peak splitting value was observed to be 7.7 eV, good consistent with the literature value for V(IV) oxide (Biesinger et al., 2010). This precipitate was also responsible for the imbalance of reduced V(V) and available V(IV) in the solution (Fig. 1) as in the pH range of natural waters the solubility of V(IV) is much smaller and it is strongly adsorbed on particles and forms stable complexes with organic (Ortiz-Bernad et al., 2004). This indicated that the microbial V(V) reduction with high efficiency in present study resulted in the successful removal of vanadium from the groundwater via in situ bioreduction followed by precipitation of a vanadium-bearing mineral or sorption of vanadium. Our results demonstrated that promoting reduction of highly mobile and toxic V(V) to less mobile and toxic V(IV) by immobilized mixed anaerobic culture could be a promising remediation strategy for immobilizing vanadium, thereby removing it from contaminated groundwater.

3.2. Influence of operating factors

With initial COD concentration of 800 mg/L, conductivity of 12 mS/cm and pH of 7.0, four levels of initial V(V) concentrations (50 mg/L, 75 mg/L, 150 mg/L, 300 mg/L) were conducted. It could seen from Fig. 2a that most of V(V) was removed gradually within the 12 h operating period. Especially at 50 mg/L, the V(V) concentration in the effluent was below the requirement (1.0 mg/L) of the Discharge standard of pollutants for vanadium industry in China (GB 26452-2011). With initial concentrations increased, the removal amount of total V(V) increased accordingly, but the removal efficiencies decreased. Exorbitant initial V(V) concentrations could suppress the anaerobic microbes’ activities, thus lowering the removal efficiencies. Previous report indicated that bacterial species were tolerant to V(V) in the range of 110 mg/L to 230 mg/L, with a gradual decrease in their colony/cell counts when V(V) concentration gradually increased (Kamika and Momba, 2012). Our results were consistent with this finding and removal efficiency significantly decreased when initial V(V) concentration increased to 300 mg/L, though there had also been electron donor available in the aqueous solution as the residual COD under this condition.

As the activities of dissimilatory metal reduction bacteria were affected by the amount of the electron donors and carbon sources, different initial COD concentrations (200 mg/L, 800 mg/L, 1200 mg/L, 1600 mg/L) were studied, with initial V(V) concentration of 75 mg/L, conductivity of 12 mS/cm and pH of 7.0. As could be seen from Fig. 2b, an appropriate increase in COD resulted in the improvement of V(V) reduction, but its efficiencies decreased when further increasing COD concentrations. The highest V(V) reduction was recorded with the initial COD concentration of 800 mg/L. As approximate 500 mg/L of COD was consumed for microbes to reduce 75 mg/L of V(V) observed in present study, too high or too low initial COD concentrations would suppress V(V) reductions. There would be no enough electron donors and carbon sources to support the microbes growth as well as V(V) reduction when the initial COD concentration was set at 200 mg/L. As fermentation substrate (ie. glucose) was employed in present study, when the COD increased substantially, anaerobic fermentation process with methane production would dominate in the bioreactor and compete electrons with dissimilatory metal reduction process, thus decreasing V(V) reduction, which had also been observed in substrate comparison studies (Freguia et al., 2008).

Fig. 2c illustrated the effects of pH (5.4, 6.2, 7.0, 7.8) on the V(V) reduction with initial V(V) concentration of 75 mg/L, COD of 800 mg/L and conductivity of 12 mS/cm. Vanadium reductases could survive in the tested pH values as V(V) was gradually removed under all the conditions, indicating that microbial V(V) reduction could occur in a relatively wide range of pH. The removal efficiency was much higher under neutral condition than that under acidic or alkaline one. According to Bell et al. (2004), the pH effects played a major role in the toxicity of vanadium salts in the nutrient broth. This confirmed findings that pH changes could affect the tolerance limits of test organisms to V(V) in mixed liquor (Freguia et al., 2008). At high pH, the solubility of some
metals decreased, while at low pH, those metals were found as free ionic species in aqueous solutions and were capable to express their toxicities. As the toxicities of V(V) differentiated, its removals were also varied.

Different conductivities (10 mS/cm, 12 mS/cm, 15 mS/cm, 19 mS/cm) were also performed with initial V(V) concentration of 75 mg/L, COD concentration of 800 mg/L and pH of 7.0. Gradual decreases of V(V) concentration were observed during the operation, while V(V) reduction was first enhanced considerably with the increase of conductivities up to a threshold value (12 mS/cm here) and then it was weakened substantially with further increase of conductivities (Fig. 2d). Proper conductivities could accelerate electron transfer from glucose to V(V) via bacteria due to the presence of additional ions. The decrease of V(V) reduction with higher conductivities could attribute to the reason that the high salinity could poison the anaerobic microbes as reported previously (Zhang et al., 2010).

V(V) and anaerobic microbes could coexist and microbial reduction of V(V) to V(IV) by immobilized mixed anaerobic sludge was effective for bioremediation of V(V) contaminated groundwater. To promote the efficiency of proposed system for in situ bioremediations, certain environmental conditions could be optimized based on above operating factors studies and the influences of typical components of groundwater, such as diluting the V(V) contaminated groundwater with widely existing brackish groundwater to proper initial V(V) concentration with improved conductivity.

### 3.3. Identification of the involved microbes

#### 3.3.1. Richness and diversity of bacteria phylotypes

21635 and 16126 sequences were obtained for the inoculated sludge and the bioreactor respectively, with average length of 395 bp. We also obtained 241 (inoculated sludge) and 110 (Bioreactor) operational taxonomic units (OTUs) individually at a 3% distance. However, new bacterial phylotypes continued to emerge even after 12,000 reads sampling with pyrosequencing as exhibited by rarefaction curve (Fig. 3). Fig. 3 also showed the diversity of bacteria in the bioreactor was significantly reduced after 3 months accumulation compared with the seed sludge due to the V(V) toxicity to microbes. Moreover, the sum of total observed OTUs in both communities was 299, but only 52 OTUs or 17.4% of the total OTUs were shared by them, indicating the microbial population structure evolved with V(V) acclimatization. The total numbers of OTUs estimated by Chao1 estimator were 258 (inoculated sludge) and 135 (Bioreactor) with infinite sampling, which also implied the decrease of community richness in the bioreactor.

To promote the efficiency of proposed system for in situ bioremediations, certain environmental conditions could be optimized based on above operating factors studies and the influences of typical components of groundwater, such as diluting the V(V) contaminated groundwater with widely existing brackish groundwater to proper initial V(V) concentration with improved conductivity.

#### 3.3.2. Taxonomic complexity of the bacterial community

To identify the phylogenetic diversity of bacterial communities in the inoculated sludge and the bioreactor, qualified reads were assigned in Fig. 4, which showed the relative bacterial community abundances at the phylum level. The community of the inoculated sludge showed a dramatically high diversity, reflected by the fact that 25 identified bacterial phyla were detected. Even so, 12.0% of the total reads were not classified at the phylum level, suggesting that these bacteria were unknown. The high-throughput Illumina pyrosequencing results indicated its bacterial library mainly included Bacteroidetes (occupying 30.9% of total bacterial clones), Proteobacteria (20.9%), Spirochaetae (7.8%), Nitrosirae (4.7%), Chloroflexi (4.6%). Compared with the inoculated anaerobic sludge, characteristics of microbial population of the bioreactor changed significantly, with only 14 identified phyla and unclassified bacteria of 2.7% (Fig. 4). The percentages of Bacteroidetes, Spirochaetae and Proteobacteria decreased to 10.1%, 4.3% and 1.5%, respectively, illustrating that they were more sensitive to V(V) toxicity. The value of Firmicutes was increased extremely about 20 times compared with the seed sludge due to the presence of additional ions. The decrease of V(V) reduction with higher conductivities could attribute to the reason that the high salinity could poison the anaerobic microbes as reported previously (Zhang et al., 2010).

V(V) and anaerobic microbes could coexist and microbial reduction of V(V) to V(IV) by immobilized mixed anaerobic sludge was effective for bioremediation of V(V) contaminated groundwater. To promote the efficiency of proposed system for in situ bioremediations, certain environmental conditions could be optimized based on above operating factors studies and the influences of typical components of groundwater, such as diluting the V(V) contaminated groundwater with widely existing brackish groundwater to proper initial V(V) concentration with improved conductivity.

#### Bacterial community compositions at phylum level revealed by pyrosequencing of inoculated sludge and bacterial communities in the bioreactor.

![Fig. 4. Bacterial community compositions at phylum level revealed by pyrosequencing of inoculated sludge and bacterial communities in the bioreactor.](image-url)

Inoculated sludge | Bioreactor
---|---
**Acidobacteria** | **Acidobacteria**
**Bacteroidetes** | **Bacteroidetes**
**Chlorobi** | **Chlorobi**
**Chloroflexi** | **Chloroflexi**
**Deferribacteres** | **Deferribacteres**
**Elusimicrobiia** | **Elusimicrobiia**
**Firmicutes** | **Firmicutes**
**Gallocardiales** | **Gallocardiales**
**GOUTA4** | **GOUTA4**
**Hyd24-12** | **Hyd24-12**
**KA4** | **KA4**
**Lentisphaerae** | **Lentisphaerae**
**Nitrospira** | **Nitrospira**
**Planctomycetes** | **Planctomycetes**
**Proteobacteria** | **Proteobacteria**
**Spirocetetes** | **Spirocetetes**
**Tetragenobacterales** | **Tetragenobacterales**
**TM7** | **TM7**
**Tenericutes** | **Tenericutes**
**Thermotogae** | **Thermotogae**

*Fig. 4. Bacterial community compositions at phylum level revealed by pyrosequencing of inoculated sludge and bacterial communities in the bioreactor.*
Table 2
The percentages of sequences identified to different phylogenies for the inoculated sludge and in the bioreactor.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Genus</th>
<th>Inoculums (%)</th>
<th>Bioreactor (%)</th>
<th>Phylum</th>
<th>Class</th>
<th>Genus</th>
<th>Inoculums (%)</th>
<th>Bioreactor (%)</th>
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<tr>
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<td>Gelria</td>
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<tr>
<td>Nitrospirae</td>
<td>Nitrospira</td>
<td>Nitrospira</td>
<td>4.68</td>
<td>0.01</td>
<td>Others</td>
<td></td>
<td></td>
<td>35.50</td>
<td>16.40</td>
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current production using molecular and culture-dependent methods (Wrighton et al., 2008).

High-throughput sequencing methods had the potential to be effective means for better understanding of the microorganisms in various environmental processes. Standing on the genus level could further infer the functions of the involved communities (Table 2).

V(V) reducing microbes could function well with energy from the oxidation of organic compounds under anaerobic environment. Enterobacter of Proteobacteria able to realize dissimilatory reduction of V(V) as the one isolated from a deep gold mine in South African was dramatically enriched in the bioreactor, with its percentage increasing from 0.05% to 0.37% (van Marwijk et al., 2009). In addition, some enhanced species could also function to V(V) reduction despite few direct reports were released before, as dissimilatory reduction of metals was common in bacterial world. For instance, the predominating species Lactococcus genus of Firmicutes in the biofilm (59.36%) could reduce and precipitate silver in its metallic form (Sintubin et al., 2009). The enriched Spirochaeta of Spirochaetes could realize Uranium (VI) reduction mainly through an enzymatic catalysis (Martins et al., 2010), while the newly generated Eubacterium of Firmicutes had been demonstrated to degrade DDT in pure culture as well as natural soil (Cao et al., 2012). V(V) reductase activity was membrane-associated and was coupled with the oxidation of NADH to the reduction of V(V), thus these mentioned species might also play roles in V(V) reduction.

There were also lots of fermentative microorganisms found in the bioreactor as the fermentation substrate glucose was employed and V(V) reducing microbes could conserve energy to support their growth with various electron donors such as hydrogen and organic acids. Particularly, the significantly enhanced Paludibacter of Bacteroidetes from 1.34% to 8.46% belonging to fermentative bac-

of Firmicutes and Victivallis of Lentisphaerae in the bioreactor were reported members of the family fermentative micro-organisms capable of producing acetate and hydrogen (Micieli ill et al., 2012). Both the newly appeared Acetobacterium, and Oscillibacter of Firmicutes were also proficient in degrading part of organic matters to small molecule acid such as acetate (Peters et al., 1998). These microbes interacted and their products might improve the activities of V(V) reducing microbes regarding to V(V) reduction.

In another aspect, some specific bacteria related to extracellular electron transfer were also detected in the bioreactor. The emerging Clostridium of Firmicutes (0.26%) was reported as biocatalyst to transfer electrons for microbial electricity generation (Wong et al., 2014). The newly generated Dysgonomonas of Bacteroidetes (0.23%) was proved that it could transfer electrons from solution to solid electrode directly in MFCs (Zhao et al., 2008). Enterobacter of Gammaproteobacteria was also involved in hydrog-

erin respiration with electrochemical activity, thus generating bio-
ectricity (Rezaei et al., 2009). This implied that these electrochemically active bacteria had a high tolerance to V(V) toxicity and their existence could promote V(V) reduction.

Summarily, present study realized detoxification of V(V) in groundwater by immobilized mixed anaerobic culture. V(V) was reduced to V(IV) that resulted in formation of a vanadium precipitate as indicated by SEM, EDX and XPS results. The efficiencies were enhanced as composite microbes were presented in the anaerobic sludge and they could achieve intracellular and membrane-associated V(V) reduction through their interaction, compared with studies carried out on pure cultures (Carpentier et al., 2003, 2005; Ortiz-Bernad et al., 2004). New functional microorganisms for V(V) reduction as Lactococcus genus of Firmicutes and Spirochaeta of Spirochaetes were found and their performances were deduced. Intensive studies would be conducted to further reveal correlations between these new species and V(V). Moreover, anaerobic sludge could be washed and then mechanically dehydrated to prevent it potentially contaminating groundwater. After that, it could be added in the permeable reactive barrier, the most widely employed form for groundwater remediation to control V(V) pollution effectively.

4. Conclusions

Vanadium (V) reduction was successfully achieved by immobilized mixed anaerobic sludge. V(V) removal efficiency decreased with the increase of initial V(V) concentration, while its value increased first and then decreased with the increase of initial COD concentration, pH and conductivity. Microbial community analysis indicated the decreased diversity. Significantly enhanced Lactococcus and Enterobacter with oxidation of lactic and acetic acids from fermentative microorganisms such as the enriched Paludibacter and the newly appeared Acetobacterium, Oscillibacter were responsible for V(V) reduction. This study is helpful to detect new functional species for V(V) reduction and build promising processes for bioremediation of V(V) contamination.

Acknowledgements

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