Simultaneous microbial and electrochemical reductions of vanadium (V) with bioelectricity generation in microbial fuel cells

Baogang Zhang, Caixing Tian, Ying Liu, Liting Hao, Ye Liu, Chuanping Feng, Yuqian Liu, Zhongli Wang
School of Water Resources and Environment, China University of Geosciences Beijing, Beijing 100083, China
Key Laboratory of Groundwater Circulation and Evolution (China University of Geosciences Beijing), Ministry of Education, Beijing 100083, China

hIGHLIGHTS
• Vanadium (V) is reduced simultaneously in anode and cathode chambers of MFCs.
• Bioelectricity is generated at the same time during this process.
• Vanadium (IV) is the main reduction product and can subsequently precipitate.
• Deltaproteobacteria, Bacteroidetes and Spirochaetes are the functional species.

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A B S T R A C T
Simultaneous microbial and electrochemical reductions of vanadium (V) with bioelectricity generation were realized in microbial fuel cells (MFCs). With initial V(V) concentrations of 75 mg/l and 150 mg/l in anolyte and catholyte, respectively, stable power output of 419 ± 11 mW/m² was achieved. After 12 h operation, V(V) concentration in the catholyte decreased to the value similar to that of the initial one in the anolyte, meanwhile it was nearly reduced completely in the anolyte. V(IV) was the main reduction product, which subsequently precipitated, acquiring total vanadium removal efficiencies of 76.8 ± 2.9%. Microbial community analysis revealed the emergence of the new species of Deltaproteobacteria and Bacteroidetes as well as the enhanced Spirochaetes mainly functioned in the anode. This study opens new pathways to successful remediation of vanadium contamination.

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1. Introduction
Vanadium (V) is a widespread environmental contaminant from either natural or industrial sources as it widely exists in the Earth’s crust and is extensively employed in modern industry including metallurgy and petroleum refining (Carpentier et al., 2003; Zhang et al., 2009). It is moderately toxic to animals and becomes toxic to animal cells at concentrations greater than 1–10 μg/l (Yelton et al., 2013). Vanadium naturally occurs in three oxidation states: V(III), V(IV), and V(V), among which V(V) is the most common valence for industrial use. V(V) is more toxic than other species and is known to cause oxidative cell damage (Safavi et al., 2000). Previous methods for V(V) removal from wastewater included the use of cement immobilization (Bhatnagar et al., 2008), precipitation (Navarro et al., 2007), and adsorption (Naeem et al., 2007).

However, the cost-effectiveness of such methods is questionable. Moreover, the reduction of V(V) to V(IV) represents a major way to reduce the impact of vanadium on living systems and microbial reduction of V(V) has been recognized as an economical means (Carpentier et al., 2003), but most current studies on microbial V(V) reduction are only carried out in pure cultures such as Geobacter metallireducens, Shewanella oneidensis and their efficiencies should be improved (Yelton et al., 2013).

Microbial fuel cells (MFCs) are devices that use bacteria as catalysts to oxidize organic or inorganic matter and generate electricity (Logan et al., 2006). MFCs are capable of simultaneous biological electricity generation and wastewater treatment, so offer an economic pathway to sustainable energy utilization (Zhuang et al., 2010). Electron acceptors in the cathode compartment play an important role in MFC performance (Zhao et al., 2006) and our previous research has reported the application of V(V) as the alternative cathode electron acceptor for the first time, realizing the simultaneous reduction of V(V) (Zhang et al., 2009), due to its higher electrochemical redox potentials (Eq. 1). As
the anode chamber of MFCs was operated in anaerobic environment and V(V) can also be reduced through co-metabolism pathway under anaerobic condition (Yelton et al., 2013), it can also be considered for biological V(V) pollution control. Therefore combinations of electrochemical and biological V(V) reductions with respective advantages as well as energy recovery can be realized in MFCs.

\[
\text{VO}_4^{2-} + 2\text{H}^+ + e^- \rightarrow \text{VO}^{2+} + \text{H}_2\text{O} \quad \text{E}^\circ = 0.991\text{V}
\]  

In the present study, the feasibility of V(V) reductions both in anode and cathode chambers of the MFCs simultaneously was investigated. Mixed anaerobic culture was employed to improve the efficiencies of microbial V(V) reduction and electricity generation. Performances of the MFC under different combinations of initial V(V) concentrations in anode and cathode were studied. The involved bacteria as the key factor of electricity generation and V(V) reduction in this system was also monitored and analyzed. The subsequent processes of V(V) treatment and recovery were also explored. The results were helpful to reveal the mechanisms of V(V) reduction and recovery with power outputs based on MFC technology.

2. Methods

2.1. Double chamber MFCs construction and electrolyte conditions

The four double chamber MFCs built in cylindrical geometry chambers were employed (Zhang et al., 2009). The anode and cathode chambers were separated by a proton exchange membrane (Nafion117#. Dupont, USA) and were airproofed by continuously sparging with N2 to prevent oxygen in the ambient air. The anodic and cathodic electrodes both made of carbon fiber felt with dimensions of 40 × 40 × 10 mm were connected by copper wires with the external resistance of 100Ω. Each chamber was filled with 250 ml electrolyte. The anode solution contained the following components (per l): 0.75 g of C6H12O6; 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). V(V) was added into above solution in the form of NaVO3 with the given concentration. The initial pH of anolyte was maintained about 7 with the addition of phosphate buffer solution. The cathode solution was simulated vanadium containing wastewater with V(V) concentrations of 150 mg/l and pH of 2 adjusted by HCl (1:1). The MFCs were connected to the data acquisition system (Measurement Inc, USA) by copper wires, and the voltage recorded at 5 min intervals.

2.2. Operation of the double chamber MFCs

The four MFCs were inoculated with 25 ml anaerobic granular sludge obtained from an up-flow anaerobic sludge blanket (UASB) reactor treating high strength sulfate wastewater. After they were well developed, the suspended sludge was removed from the anode compartment. Two fully developed MFCs were conducted with initial V(V) concentrations in anolyte and catholyte of 75 mg/l and 150 mg/l, respectively, which were first incubated with the electrolysates described above, under condition of close circuit for three months. Electrolyte was refreshed each day for domestication. Then these MFCs were operated to evaluate V(V) reductions both in the anode and cathode chambers with electricity generation under different initial V(V) concentration combinations, i.e. 50 mg/l vs. 120 mg/l, 75 mg/l vs. 150 mg/l, 100 mg/l vs. 200 mg/l, respectively (in anolyte vs. in catholyte, in turn). The 12 h fed-batch mode was chosen as most V(V) was reduced within that time. The MFCs were also operated under open circuit under specific condition to consider the biological and chemical V(V) reductions individually, with initial V(V) concentrations in anolyte and catholyte of 75 mg/l and 150 mg/l, respectively. Another two MFCs were also operated as control sets wherein, without the addition of V(V) in the anolyte, while the initial V(V) concentration in catholyte was 150 mg/l as well. To conform the synergistical treatment of V(V) containing wastewater in cathode and anode chambers of the MFCs in sequence, in the end of a typical cycle, the exhausted catholyte was fed into the anode chamber as the new anolyte with pH as well as other elements adjustment and the MFCs operated for another 12 h with fresh electrolyte in the cathode. Subsequently, this solution was drained out and the subsequent processes of V(V) treatment and recovery were explored by chemical precipitation, as V(IV) has minimum solubility at pH 6 (Charrier et al., 1996). pH was adjusted by adding NH3·H2O (1:1). At last, microbes on the anodic electrodes in these two groups of MFCs were tested and analyzed after another 3 months accumulation. PCR and 16S rDNA gene sequences analysis were performed to obtain the strains information and their effects on V(V) reduction and energy recovery. Two MFCs in each group were operated under identical conditions and the average results were recorded. All the experiments were carried out at room temperature (25 ± 2°C).

2.3. Electrochemical analytical methods and microbiological analysis

Chemical oxygen demand (COD) was measured by fast airtight catalytic decomposition method. Spectrophotometric methods were chosen to measure the reduction of V(V) and generation of V(IV) (Safavi et al., 2000; Ensafi et al., 1999). Other species of vanadium such as V(III) and V(II) were not measured as they were rarely generated, as indicated in our previous study (Zhang et al., 2009). Total vanadium was determined by ICP-MS (Thermo Fisher X series, Germany). pH was measured using a pH-201 meter (Hanna, Italy).

Anodic and cathodic half-cell potentials were monitored by placing Ag/AgCl reference electrodes in both compartments. Polarization curves were employed to obtain the maximum power density by varying external resistances from 5000 Ω to 10 Ω using a resistor box and MFCs operated at least twice under each resistance to ensure the repeatability of power outputs. Current density and power density were normalized by the cathode area (the single-side projected area). Coulombic efficiency (CE) was calculated as reported previously (Zhang et al., 2009).

The surface morphology of the anodic electrode was examined by scanning electron microscopy (SEM) (Quanta, FEI Co., Hillsboro, OR, USA) after the whole experiment. Energy dispersive X-ray (EDX) analysis was performed at the same time for elemental analysis of the anode surface. Deposits generated after precipitation process were determined by 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 anode surface of the MFCs (Sample M) and the control sets (Sample C). 16S rDNA gene fragments of bacteria were extracted, amplified, quantified and the phylogenetic analysis was performed as described before (Zhang et al., 2013). Sequences reported in this paper have been submitted to GenBank with accession numbers from KJ466920–KJ466960.

3. Results and discussion

3.1. Power outputs of the MFCs

The double chamber MFCs were initially filled with anolyte containing 750 mg/l of glucose and 75 mg/l V(V), and 150 mg/l of V(V)
at pH 2 as electron acceptor in the cathode chamber. The control sets were operated with the identical electrolyte except the addition of V(V) in the anode solution. The performance of the MFCs was stable and the voltage outputs maintained 420–460 mV with 100 Ω external resistance in a typical operating cycle (12 h). The voltage output obtained in the present study was promising compared with previous research findings for double chamber MFCs (Mohan et al., 2008). The cathode potential (320–270 mV, vs. Ag/AgCl) was also similar with that of K_{3}Fe(CN)_{6} solution (100 mM) and oxygen (both less than 400 mV) cathodes (Zhuang et al., 2010; Dutta et al., 2008), due to the relatively higher electrode potentials of V(V) under high concentration and low pH according to the Nernst equation. The voltage outputs of the present MFCs were also slightly affected due to a little increase in the anode potentials (−180 mV to −200 mV, vs. Ag/AgCl), compared with previous study (−250 mV) (Zhang et al., 2009) and the control sets (−230 mV to −250 mV), as the addition of oxidative states V(V) in the anode substrate could increase anode potentials.

Polarization curves were obtained with closed-circuit MFCs (Fig. 1). The highest power output was 418.6 ± 11.3 mW/m² at a current density of 1143.8 ± 22.4 mA/m², which was higher than that of cathode-aerated MFCs previously reported by Chung et al. (2011). Differences in solubility and mass transfer between the two types of electron acceptors (oxygen and V(V)) could influence power generation. V(V) had similar standard electrode potentials with oxygen, but its solubility was much greater than oxygen, thus lowering the mass transfer resistance and providing abundant electron acceptors in the cathode electrolyte. Though the level of power generation obtained in the present study was slightly below that reported by Zhang et al. (2009) which generated 572 mW/m² with sulfide lowering anode potential and the control sets without V(V) raising anode potential (470.5 ± 19.8 mA/m²), the simultaneous V(V) removal in the anode chamber could compensate this slight decrease in power outputs as more hazardous pollutants could removed within the same operating time, which would be further discussed in Section 3.2.

The calculated CE based on COD of the MFCs was about 24.8%, a bit lower than that (25.8%) of conventional MFCs employing glucose as fuel (Zhang et al., 2012), indicating that not all electrons from glucose consumption were transferred to the anode for electricity generation. Besides the own consumption during bacteria metabolism, a portion of generated electrons (about 3.6%) were consumed to reduce V(V) in the anode chamber, which could also be deduced from the control sets.

Another two initial V(V) concentration combinations, i.e. 50 mg/l vs. 120 mg/l, 100 mg/l vs. 200 mg/l (in anolyte vs. in catholyte, in turn) were also tested. Energy could also be recovered under these conditions during the same operating cycle (12 h) (Fig. 1). It could be seen that maximum power densities increased with the increase of the initial V(V) concentration. As the addition of V(V) could raise the electrode potential, both for anode and cathode. The phenomenon indicated that the power outputs were dominated by cathode potentials in present study. The anode chamber worked under anaerobic environment and anaerobic bacteria could maintain relatively lower redox potential, thus resisting the possible increase of anode potential in virtue of addition of V(V) (Logan et al., 2006).

3.2. Simultaneous microbial and electrochemical reductions of V(V)

V(V) concentrations were monitored during the operation of the MFCs, with initial values of 75 mg/l and 150 mg/l in the anolyte and catholyte, respectively. Gradual reduction of V(V) over the duration of the test was observed both in the anode and cathode chambers (Fig. 2). All the electrons for V(V) reduction originated from microbial glucose oxidation process. Ordinarily, parts of them (24.8% as CE indicated) were transferred to anode electrode by electrochemically active bacteria and then flowed to the cathode electrode through external circuit for cathodic electrochemical V(V) reduction, which we had reported before (Zhang et al., 2009). Another portion of electrons (3.6%) were directly transferred to V(V) in anolyte immediately after their generation and V(V) was thus biologically reduced in the anode chamber. After the 12 h operation, V(V) concentration in the catholyte decreased to the value similar to the initial one in the anolyte, meanwhile V(V) was nearly reduced completely in the anolyte, which provided the possibility of synergistic treatment of V(V) containing wastewater. V(V) was also monitored and its concentration increased accordingly. Previous study (Zhang et al., 2010) indicated electrochemical V(V) reduction would be restricted when the operation prolonged as the generated V(V) could be further reduced instead of V(V) reduction (Zhang et al., 2010). This present achievement was outstanding with preventing the similar problem as V(V) could be simultaneously reduced in the catholyte electrochemically and in anolyte biologically. pH of the exhausted anolyte dropped from 6.97 to 6.72, while it rose from 2.14 to 2.28 in the catholyte. Meanwhile, about 300 mg/l COD were removed synchronously in the anode during the typical operating cycle. The above results indicated that the two chambers of the MFCs could remove V(V) synergistically, with pretreatment in cathode chamber and further

![Fig. 1. Polarization curves and power outputs obtained for MFCs and the control sets. Initial V(V) in Combination 1: 50 mg/l vs. 120 mg/l; in Combination 2: 75 mg/l vs. 150 mg/l; in Combination 3: 100 mg/l vs. 200 mg/l (in anolyte vs. in catholyte, in turn).](image1)

![Fig. 2. Time histories of V(V) concentrations in the electrolyte under different combinations during the 12 h operation of the MFCs. Initial V(V) in Combination 1: 50 mg/l vs. 120 mg/l; in Combination 2: 75 mg/l vs. 150 mg/l; in Combination 3: 100 mg/l vs. 200 mg/l (in anolyte vs. in catholyte, in turn).](image2)
treatment in anode chamber sequentially. Common vanadium containing wastewater is often produced in large quantities with high V(V) concentration (100–400 mg/l) and low pH (2–3) from vanadium mining and vanadium pentoxide manufacture, and traditionally treatment process is chemical reduction, precipitation and then pH adjustment before its discharge (Safavi et al., 2000). By employing MFC technology proposed in present study, reduction and precipitation chemicals could be left out, with electrochemical reduction in the cathode chamber directly. The pH adjustment was performed as usual and the exhausted catholyte mixed with domestic wastewater (containing organics) which also appeared in the vanadium ore district was further treated in the anode chamber, thus promising alternative for V(V) treatment was provided.

In another aspect, these two microbial and electrochemical effects were also studied separately. In the control sets, V(IV) decreased to 55.4 mg/l from 150 mg/l in the catholyte with relatively quicker speed than the MFCs during the same operating cycle, due to the slightly larger current. However, the ability of the anode for biological V(IV) reduction was not played, which resulted in less total V(IV) reduction within the same time compared with the MFCs. Moreover, Experiment was also carried out with the MFCs operated in open circuit. V(IV) reduction in the catholyte was not observed without electrons from the anode, while V(IV) concentrations were changed from 75 mg/l to 13.5 mg/l during the same operating cycle. This value was above the V(IV) concentration in the exhausted anolyte from the MFCs operated during the same operating cycle. This value was above the V(IV) concentration in the exhausted anolyte from the MFCs operated in closed circuit, implying that functional microbes for V(IV) reduction could be enhanced when electron completions with solid anode electrode happened with the presence of sufficient electron donors, which had also been observed in the anode chamber of MFC by Li et al. (2009). The MFCs could reduce more V(IV) in closed circuit than those in open circuit as well within the same operations. This enhancement of V(IV) reduction demonstrated the feasibility and advantages of synergy effects of anode and cathode of the MFCs for V(IV) removals, though their energy recovery was slightly affected as the addition of V(IV) in the anode chamber.

Another two initial V(IV) concentration combinations, i.e. 50 mg/l vs. 120 mg/l, 100 mg/l vs. 200 mg/l (in anolyte vs. in catholyte, in turn) were also examined. Similar downward tendencies of V(IV) concentration were observed (Fig. 2). It could also be seen that the decreasing trend slowed down with the increase of initial V(IV) concentrations (Fig. 2). Moreover, all V(IV) in the catholyte could decreased to the initial concentration of V(IV) in the anolyte, while anodic V(IV) could be reduced completely at the end of the 12 h operating cycle. This offered multiple selectivity for V(IV) reduction in MFCs based on the characteristics of raw wastewater, exhibiting the promising application prospect for vanadium pollution treatment.

3.3. Identification of the involved microbes

3.3.1. Acquisition of the microbes information

After 3 months operation, the MFCs exhibited stable electricity outputs and V(IV) removals, suggesting that an active bacterial consortium capable of electricity production and V(IV) reduction from glucose oxidation was established in the anode chamber of MFCs. In this study, 56 and 99 white colonies with inserted small-subunit ribosomal genes were randomly chosen to construct bacterial libraries, respectively. The results of the in situ PCR indicated that there were 14 genera covered in Sample M (microbes attached on the anode surface of the MFCs) and 30 in Sample C (microbes attached on the anode surface of the control sets). The 165 rDNA gene sequences of the Sample C bacterial library fell into mainly 12 phylogenetic divisions (Fig. 3a), mainly including Firmicutes (occupying 45% of total bacterial clones), Chlorobi (21%), Deltaproteobacteria (11%), TM 7 (5.1%), Bacteroidetes (4%). Compared with the inoculated anaerobic sludge, characteristics of microbial population for Sample C did not changed significantly, but the percentage of Deltaproteobacteria increased with the Controls’ functions of electricity generation enhancing, with another two newly generated bacteria i.e. Armatimonadetes (2%) and Lentisphaerae (1%) (Zhang et al., 2013; Wang et al., 2012). However, the Bacteroidetes decreased despite some of them belonging to electrochemical activity bacteria such as Bacteroides sp. due to lack of sulfide as co-substrate in the anode chamber (Zhang et al., 2013). Moreover, there were only 8 phylogenetic divisions of the Sample M bacterial library, while its colony structure had obviously altered over domestication (Fig. 3b). Betaproteobacteria, Lentisphaerae, Armatimonadetes, BRC1, TM7, uncultured bacteria disappeared after 3 months domestication. In the meantime, Chloroflexi (3.7%), Gammaproteobacteria (3.7%) appeared on the anode surface of the MFCs, while the percentage of Spirochaetes increased from 1.0% to 11.1%. Firmicutes and Chlorobi were also the first and second predominant bacteria in Sample M, just as in Sample C, but their potions decreased as few bacteria fell in these two phyla were reported with electrochemical activity or metal reduction ability. These indicated that structures of the bacteria community had evolved as adapting to the new conditions during the operation. Some specific species might be responsible for V(IV) reduction in the MFCs and thus should be further investigated.

Fig. 3. Proportions of each phylotype on the anode surface of the controls (a) and the MFCs (b) clone libraries.
3.3.2. Deduction of the microbes effects

To know more about specific roles of bacteria in electricity generation and V(V) reduction in the anode chamber of the MFCs, a neighbor-joining tree was constructed with these and related sequences from the GenBank database (Fig. 4). Some critical species responsible for the electricity generation and V(V) reduction were discovered, which also exhibited specific characteristics in the proposed MFCs.

Electrochemically active bacteria were conducive to sustainable electricity generation in MFCs. Bacterial species classified as the Deltaproteobacteria (11.2%), Bacteroidetes (4.1%), Armidimonadetes (2%) and Lentisphaerae (1%) were involved in electricity generation in the control sets, which had also been indicated in early MFCs studies (Zhang et al., 2013). However, Armidimonadetes and Lentisphaerae disappeared, with sight decrease of Deltaproteobacteria (11.1%) and Bacteroidetes (3.7%) in the proposed MFCs, indicating these electrochemically active bacteria were sensitive to the toxicity of V(V). The decrease of power outputs of the MFCs could also be owed to these changes. Besides members of Deltaproteobacteria (Desulfobulbus sp. and Desulfovibrio desulfuricans) with electrochemically activity (Holmes et al., 2004; Cooney et al., 1996), others contributing to electricity generation were also found in the proposed MFCs, especially the newly generated Gammaproteobacteria. For example, Enterobacter sp., had also been reported to degrade cellulose and generate electricity simultaneously in MFCs (Rezaei et al., 2009). Research had also been reported for the present Tolumonasosonensis and its electrochemical characteristics (Luo et al., 2013). This implied that these electrochemically active bacteria had a high tolerance for V(V) toxicity, which were propitious to its reduction with electricity generation.

Specific microbes could obtain energy for growth by coupling the oxidation of organic compounds to the reduction of V(V) under the anaerobic environment. It was previously reported that various microorganisms could reduce V(V), of most interest in this regard were Pseudomonas species of Gammaproteobacteria and Geobacter species of Deltaproteobacteria (Ortiz-Bernard et al., 2004; Antipova et al., 1998). Though these two phyla were also detected in the present study, these common microorganisms were not found, suggesting there were other species responsible for V(V) reduction. It was reported that Proteobacteria were the dominant vanadium resistant/reducing organisms (Yelton et al., 2013), and the observed Desulfovibrio sp. classified as the Deltaproteobacteria had the ability to catalyze reduction of solid V(V) oxides vanadium with mitochondrial c-type cytochromes (Lojou et al., 1998). In addition, the three newly generated phyla could also function to V(V) reduction despite few direct reports before. For instance, Enterobacter sp. of Gammaproteobacteria were able to realize dissimilatory reduction of V(V) as the one isolated from a South African deep gold mine (van Marwijk et al., 2009), where this type of microbes appeared. The uncultured Chloroflexi could anaerobically transform polychlorinated biphenyls (PCBs) by the mechanism of reductive dechlorination (Fagervold et al., 2005). Sphaerochaeta sp. of Spirochaetes reached uranium (VI) reduction mainly through an enzymatic catalysis (Martins et al., 2010). These two species might also be conducive to V(V) reduction as the reductases were widespread and V(V) reductase activity was membrane-associated as well as coupled the oxidation of NADH to the reduction of V(V), which was a common process in microbial metabolism.

In another aspect, V(V) reducing bacteria could conserve energy to support their growth with hydrogen, various sugars, and organic acids as the electron donor. As glucose was selected as initial electron donors and its oxidation products were various with functions of fermentation bacteria as Anaerobacillus burkinensis and Lactococcus Firmicutes, the V(V) reducing bacteria also exhibited much...
diversity. There was no Fe$^{3+}$ present in the inoculum or the anolyte, and there was little acetate accumulation during the operation, thus dissimilatory metal-reducing microorganisms related to V(V) reduction were not found, as the growth of these kinds of V(V) reducing bacteria such as Geobacter species required Fe$^{3+}$ and acetate (Ortiz-Bernad et al., 2004), while both of them were not detected in our system. Although there were also Acetobacterium species of Firmicutes in Sample M, their function mainly lied in fermenting glucose and the generated acetate might be consumed by other heterotrophic bacteria. Interestingly, microbes involved in hydrogen respiration were detected. Such as Enterobacter sp. of Gammaproteobacteria and Ruminobacillus sp. and Sphaerochaeta sp. of Spirochaetae participating in V(V) reduction with high probability could consume hydrogen as electron donor (Li et al., 2013). This indicated that hydrogen might be the direct electron donor for V(V) reducing bacteria in present study, other microorganisms fermented glucose sequentially and collaboratively. However, hydrogen was not detected during the operation as the simultaneous generation and consumption. Discrimination experiments could be carried out with specific pure microbes afterwards.

As the inoculum was anaerobic granular sludge for high strength sulfate wastewater treatment, there were also sulfur related bacteria found in Sample M (Fig. 4). Desulfovibulbus sp. and D. desulfuricans of Delta proteobacteria possessed the ability to generate electricity. Desulfovibrio sp. could reduce V(V) directly, also through sulfate reduction, while Chlorobaculum of Chlorobi and species of Epsilon proteobacteria utilize elemental sulfur, thiosulfate, sulfate, sulfite, dithionite, etc as electron acceptors with organics as the electron donors as well as the energy and carbon source (Zhang et al., 2013; Rodriguez et al., 2011). The reduction of initial sulfate from the inoculum (3.5 mg/l) and the re-oxidation of its products happened repeatedly, forming natural sulfate/sulfide mediates which accelerated electricity generation (Coney et al., 1996). Moreover, the existence of sulfide (0.13 mg/l) might promote V(V) reduction as it is easily reduced. This indicated that the exist sulfur related bacteria also played important roles in terms of power recovery and V(V) reduction in the proposed system.

3.4. Total vanadium removal and recovery from the exhausted electrolyte

After the collaborative treatment in the cathode and anode chambers of MFCs sequentially, V(V) was nearly undetected, with green precipitate generation. SEM results also proved this phenomenon as numerous particles accumulating on the anode surface. The precipitate was mainly comprised of vanadium and phosphorus, such as the green mineral sinocite [CaV$_2$(PO$_4$)$_3$(OH)$_4$]$_2$H$_2$O, which had also been reported in previous studies (Ortiz-Bernad et al., 2004; Zhang et al., 2014). After that, the exhausted anolyte was filtered through a $0.22 \mu$m pore size syringe filter unit three times to eliminate microorganisms and its pH was adjusted to 6, many exiguous particles appeared suspending in the solution. Two obvious broad V 2p XPS peaks appeared for these particles. The strongest counts peak, located at 516.8 eV, corresponds to V 2p$_{3/2}$. And the other peak located at 524.5 eV, corresponds to V 2p$_{1/2}$. The V 2p$_{3/2}$ - V 2p$_{1/2}$ peak splitting value was observed to be 7.7 eV, good consistent with the literature value for V(IV) oxide (Biesingera et al., 2010), again demonstrating that V(IV) was the main reduction product. After precipitation, the concentrations of deliquescent total vanadium in the filtrate were 34.8 mg/l, with its total removal efficiencies of 76.8 $\pm$ 2.9%, showing superiority to other biological treatment processes (Ortiz-Bernad et al., 2004; Yelton et al., 2013). Accordingly, it could be concluded that MFC based technologies are beneficial for vanadium pollution control as well as electricity generation.

4. Conclusions

Simultaneous microbial and electrochemical reductions of vanadium (V) with energy recovery were realized in MFCs. With initial V(V) concentrations of 75 mg/l and 150 m/l in anolyte and catholyte, Stable power outputs of 418.6 $\pm$ 11.3 mW/m$^2$ were achieved. After 12 h operation, V(V) concentration in the catholyte decreased to the value similar to the initial one in the anolyte, meanwhile V(V) was nearly reduced completely in the anolyte. V(IV) was the main reduction product. With its subsequent precipitation, total vanadium removal efficiencies of 76.8 $\pm$ 2.9% were realized. Molecular biology analysis revealed the newly generated Deltaproteobacteria and Bacteroidetes as well as the enhanced Spirochaetae were responsible for these processes. This study opened new pathways in the bioremediation of V(V) contamination.

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