Effects of various organic carbon sources on simultaneous V(V) reduction and bioelectricity generation in single chamber microbial fuel cells

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HIGHLIGHTS

- V(V) reduction and bioelectricity generation are realized in single chamber MFCs.
- Acetate performs best, compared with glucose, citrate and soluble starch.
- Factors affecting the performance of MFCs fed with acetate are investigated.
- Functional species are detected by high-throughput 16S rRNA gene pyrosequencing.

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ABSTRACT

Four ordinary carbon sources affecting V(V) reduction and bioelectricity generation in single chamber microbial fuel cells (MFCs) were investigated. Acetate supported highest maximum power density of 589.1 mW/m², with highest V(V) removal efficiency of 77.6% during 12 h operation, compared with glucose, citrate and soluble starch. Exorbitant initial V(V) concentration led to lower V(V) removal efficiencies and power outputs. Extra addition of organics had little effect on the improvement of MFCs performance. V(V) reduction and bioelectricity generation were enhanced and then suppressed by the increase of conductivity. The larger the external resistance, the higher the V(V) removal efficiencies and voltage outputs. High-throughput 16S rRNA gene pyrosequencing analysis implied the accumulation of Enterobacter which had the capabilities of V(V) reduction, electrochemical activity and fermentation, accompanied with other functional species as Pseudomonas, Spirochaeta, Sedimentibacter and Dysgonomonas. This study steps forward to remediate V(V) contaminated environment based on MFC technology.

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1. Introduction

Vanadium is a widely distributed abundant element in earth’s crust, with extensive applications in modern industry (Ortiz-Bernad et al., 2004). Consequently, environmental concern of vanadium arises nowadays as it is very harmful to humans and animals with high concentrations (Zhang et al., 2012). V(V) is the most common valence for industrial use and is more toxic than other species due to its oxidative damage to cells, and it is also the dominant species in oxic groundwater and soil (Safavi et al., 2000). Physical and chemical methods are often employed for V (V) removal with questionable cost-effectiveness and secondary pollution (Reul et al., 1999; Safavi et al., 2000), while microbial V (V) reduction is recognized as a promising strategy for V(V) pollution remediation, gaining considerable interests in recent years (Zhang et al., 2012). Moreover, simultaneous V(V) reduction in the anode chamber and bioelectricity generation have been achieved in microbial fuel cells (MFCs), a promising technology for pollutants control and energy recovery (Li et al., 2009; Zhang et al., 2015b). Additionally, organic carbon sources play important roles on microbial metabolism. There are great differences in the aspects of microbial richness and diversity accumulated with different organic carbon sources (Najafpour et al., 2006), which in turn affects the microbial performance and functions (Yelton et al., 2013). Nevertheless, there are limited studies focusing on the effects of organic carbon sources on V(V) reduction and bioelectricity generation in MFCs with their microbial response characteristics.

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In present study, four organic carbon sources (acetate, glucose, citrate and soluble starch) which had been separately employed previously in microbial V(V) reduction were evaluated in MFCs for V(V) removal and power outputs. Operating factors and the involved microbes were examined with optimum organic carbon source as well.

2. Methods

2.1. Experimental apparatuses and electrolyte conditions

Eight single-chamber air-cathode MFCs in cubic shape as reported in our previous study were used (Hao et al., 2015), with an effective volume of 125 ml (5 cm × 5 cm × 5 cm). Carbon fiber felt (thickness of 1 cm, length and width of 4 cm) served as anode and the cathode made of plain carbon paper (with 0.5 mg/cm² of Pt felt (thickness of 1 cm, length and width of 4 cm) was placed on the opposite site of the anode, with a projected surface area of 16 cm². A data acquisition system (PMD1208LS, Measurement Computing Corp., Norton, MA, USA) at an interval of 5 min was used to record the voltage outputs of the MFCs with 100 Ω external resistance (Zhang et al., 2012). The electrolyte contained the following components for per liter: NaH₂PO₄·H₂O (4.97 g); Na₂HPO₄·2H₂O (2.75 g); NH₄Cl (0.31 g); KCl (0.13 g); vitamin solution (1.25 ml) and 12.5 ml trace mineral element solution. V(V) was added in the form of NaVO₃·2H₂O. Each MFC was inoculated with 25 ml mixed anaerobic sludge, which was obtained from an up-flow anaerobic sludge blanket (UASB) reactor treating high strength wastewater (Beijing Yanjing Brewery Co., Ltd., China). The MFCs were divided into four groups equally and operated with acetate, glucose, citrate and soluble starch as organic carbon sources, respectively, with the same chemical oxygen demand (COD) concentration (800 mg/l unless otherwise mentioned).

2.2. Experimental procedure

All MFCs were filled with fresh electrolyte containing V(V) of 75 mg/l as well as different organic carbon sources (800 mg/l) mentioned above every 3 days for microbial domestication and this lasted for 3 months. They were well developed when maximum voltage outputs of MFCs in each group kept stable. Then power outputs and V(V) removals in a typical cycle (12 h) were evaluated with the four mentioned organic carbon sources, respectively. After that, influencing factors as initial V(V) concentrations (35 mg/l, 75 mg/l, 150 mg/l, 300 mg/l), initial COD concentrations (400 mg/l, 800 mg/l, 1200 mg/l, 1600 mg/l), conductivities (9 mS/cm, 11 mS/cm, 13 mS/cm, 15 mS/cm) and external resistances (10 Ω, 100 Ω, 500 Ω, 1000 Ω) were examined separately with the selected optimum substrate. Conductivities were adjusted with different doses of NaCl. At last, the richness, diversity and taxonomy of the involved microbes with the optimal substrate were analyzed with high throughput sequencing. All the experiments were carried out at room temperature (22 ± 2 °C). The two MFCs in each group operated under identical condition during the whole experiment. For each condition, trials were carried out in triplicate and average results from the two MFCs in the same group were reported.

2.3. Analytical methods and microbiological analysis

V(V) and V(IV) were measured by spectrophotometric methods (Ensafi et al., 1999; Safavi et al., 2000). As other species of vanadium were rarely generated, they were not measured, as indicated in the previous study (Zhang et al., 2009). COD was monitored by fast airtight catalytic decomposition method and a pH-201 meter was used to measure pH. Polarization curves of the MFCs were obtained to evaluate the relationship between voltages and currents by measuring voltages at various external resistances (ranging from 10 to 5000 Ω). Power density (PD, mW/m²) was calculated according to PD = I × U/S, where S (m²) is the geometrical area of the anode, U and I are voltage (mV) and current (A), respectively.

Ultrasonic was employed to collect the bacteria attached on the anode surface. Total genomic DNA was extracted using FastDNA® SPIN Kit for Soil (Qiagen, CA, the USA) and was pooled and amplified by PCR (GeneAmp® 9700, ABI, the USA). Then a mixture of amplicons was used for high-throughput 16S rRNA gene pyrosequencing on MiSeq (Illumina, the USA). Sequences reported in this study had been submitted to the NCBI Sequence Read Archive with its study accession number of SRP056406.

3. Results and discussion

3.1. Power outputs of the MFCs with different organic carbon sources

Polarization curves were performed with closed circuit and bioelectricity could be successfully recovered by all the MFCs with the four organic carbon sources (Fig. 1). Maximum power densities of 589.1 mW/m² for acetate, 445.6 mW/m² for glucose, 555.0 mW/m² for citrate and 390.6 mW/m² for soluble starch were obtained, respectively. These results were representative in single chamber air cathode MFCs systems previously utilizing simple organic substrates (Wang et al., 2009; Zhang et al., 2014a). This demonstrated that all the employed organic carbon sources could be effectively utilized by microbes for power production. Additionally, the power outputs decreased slightly with the addition of V(V), compared with the results from the similar MFCs fed with the same substrate i.e. glucose (Zhang et al., 2015c), due to the toxicity of V(V) to microbes (Zhang et al., 2015b). Moreover, it was reported that characteristics of the substrates could influence MFCs performance (Mahmoud et al., 2014). Maximum power densities decreased generally with the increase of the molecular weight of carbon substrate and organic aids supported higher power outputs than alcohols in principle (Fig. 1). MFCs received simpler organic compounds that could be oxidized more directly, thereby simplifying the structure and function in the biofilm anode and helping bring about higher current density (Torres et al., 2007). The key microorganisms colonized the anode of MFCs were known to use only a few simple compounds as electron donors, in particular acetate (Mahmoud et al., 2014). Acetate was reported a non-fermentative substrate as well as the major fermentation products with small
molecules organic acids such as citrate when fermentative substrate such as glucose and soluble starch was employed during anaerobic fermentation and bioelectricity generation process (Macfarlane and Macfarlane, 2003). Thereby acetate and citrate supported higher bioelectricity outputs in present systems.

3.2. V(V) reduction with different organic carbon sources

With V(V) of 75 mg/l and COD of 800 mg/l, obvious V(V) removals were observed during 12 h operation in the eight MFCs with mentioned organic carbon sources, demonstrating that the selected organics functioned well to support microbial V(V) reduction (Fig. 2). V(V) could act as electron acceptors during the anaerobic microbial metabolism and itself was reduced by microbes with the added organics as both carbon sources and electron donors (Zhang et al., 2014b). Biostimulation by organics amendment could be conductive to management of V(V) contamination. At the end of the 12 h operation, V(V) removal efficiencies of 77.6% for acetate, 66.2% for glucose, 72.1% for citrate and 62.5% for soluble starch were realized, respectively, consistent with the bioelectricity generation with these four organic carbon sources in general. The color of the medium changed from yellow–brown to blue during the operation, implying V(V) was mainly transformed into V(IV), from VO$_3^-$ to VO$_2^+$ under near-neutral conditions in present research. Additionally, green precipitate was also accumulated and conformed to be a vanadyl phosphate as the green mineral sincosite as reported before (Zhang et al., 2014b). This indicated that V(V) pollution could be mitigated through microbial reduction (Zhang et al., 2015a). Anaerobic fermentation process competed with microbial V(V) reduction process in aspect of electron capture when fermentative substrates such as glucose and soluble starch were employed, thus V(V) reduction efficiencies were relatively lower (Zhang et al., 2015a). As acetate is likely to be the major electron donor supporting anaerobic respiration in subsurface environments with outstanding V(V) removal performance (Ortiz-Bernad et al., 2004), it was designated as optimum carbon source in following experiments.

3.3. Operating factors studies with optimum carbon source

Most V(V) was removed gradually with the four gradients of initial V(V) concentrations (Fig. 3a). With the increase of initial concentration, the removal amount of total V(V) increased accordingly, however, the removal efficiency decreased. Exorbitant initial V(V) concentration could suppress the anaerobic microbes’ activities, which could lower the removal efficiency of V(V). It was reported that bacterial species were tolerant to V(V) in the range of 110–230 mg/l, and their colony/cell counts had a gradual decrease when V(V) concentration gradually increased (Kamika and Momba, 2012). When initial V(V) concentration increased to 300 mg/l, the results were consistent with this finding and removal efficiency decreased significantly. The power outputs also decreased with the increase of the initial V(V) concentrations due to its toxicity to microbes and the rise of anode potentials (Zhang et al., 2015b).

COD concentration affected the activities of dissimilatory metal reduction bacteria in aspects of the amount of carbon sources and electron donors, thereby affecting the V(V) removals and power outputs (Fig. 3b). An appropriate increase of COD resulted in the improvement of both V(V) reduction and power outputs. When COD concentration further increased, the V(V) reduction efficiency decreased slightly, with the highest efficiency with initial COD concentration of 800 mg/l. As reported previously, approximate 500 mg/l of COD was consumed for microbes to reduce 75 mg/l of V(V) (Hao et al., 2015). There would be no enough electron donors and carbon sources to support the microbes’ growth with lower initial COD concentration, while methanogenesis would compete with dissimilatory metal reduction process with higher initial COD concentration, as methanogens could also employ non-fermentative acetate as electron donor and carbon source (Ortiz-Bernad et al., 2004). Power outputs were enhanced with the increase of initial COD concentration as more electrons could be provided, consistent with study by Lu et al. (2009).

Fig. 3c illustrated that V(V) removals and energy recovery varied with different conductivities. With conductivity increasing, both V(V) removal and power outputs increased first, as electron and ions transfers could be enhanced in the aqueous solution. But when the conductivity exceeded 13 mS/cm, both V(V) removal and power outputs decreased dramatically, due to that the toxic action of high salinity poisoned the anaerobic microbes. Similar phenomenon had also been observed previously (Zhang et al., 2010).

Fig. 3d exhibited that the larger the external resistance, the higher the V(V) removal efficiency and voltage outputs. The external circuit could affect the electron transfer rate (Aelterman et al., 2008). It was reported that the resistance was the rate-determining factor at over 500 $\Omega$ under certain operating conditions (Gil et al., 2003). When the external resistance was smaller, the generated electrons from acetate could transfer to the cathode conveniently through external circuit, instead of being consumed for V(V) reduction. More electrons would be reserved in the anode chamber for V(V) reduction with larger external resistances (Zhang et al., 2010).

3.4. Identification of the involved microbes with functions

21,635 and 15,352 high-quality reads (average length of 395 bp) were recorded by the high-throughput sequencing for the inocula and microbes in the MFCs with acetate, consistent with other high-throughput studies (Lu et al., 2012). Rarefaction curves reflecting the species richness of these two samples were drawn at 3% distance, where the inocula possessed larger number of operational taxonomic units (OTUs) than the MFCs fed with acetate (Fig. 4), due to the toxicity of V(V) to microbes. With the Shannon index reflecting the microbial diversity with the distribution of each species among all the species in the community (Lu et al., 2012), the inocula had the higher diversity (Shannon 4.0) than that of the MFCs fed with acetate (2.0), indicating the change of microbial diversity with the particular substrate (V(V) and acetate) as well as the specific condition of the MFCs.

Additionally, there were 27 genotypes of phylum discovered in the inocula, indicating the rich diversity of the inoculated sludge.

![Fig. 2. Time histories of V(V) concentrations in the MFCs with four kinds of carbon sources during the 12 h operation.](image-url)
with mixed anaerobic culture (Fig. 5). However, there were only 12 genotypes of phylum found in the MFCs fed with acetate, indicating remarkable changes happened compared with the inocula. During the operation, amount of genotypes disappeared, with Actinobacteria and Firmicutes increasing significantly. As adapting to the new conditions, structures of the bacteria community had changed.

To understand more about specific roles of bacteria in MFCs with microbial V(V) reduction and bioelectricity generation, a taxonomy analysis with phylum, class and genus levels was performed (Table 1). Specific heterotrophic microbes which had the ability to reduce V(V) were detected. For example, Enterobacter of Proteobacteria able to realize dissimilatory reduction of V(V) as the one isolated from a deep gold mine in South African was enriched (van Marwijk et al., 2009). Pseudomonas of Proteobacteria having higher tolerance to V(V) was also detected (Aendekerk et al., 2002). Others could also possess the ability of reducing V(V) as their function of reducing other metals had been reported. Actinobacteria possessing Cr(VI) reducing ability was enriched (Polti et al., 2011). Comamonas of Proteobacteria could reduce Fe(III) (hydr) oxides by coupling the oxidation of electron donors and the enhanced biodegradation of 2,4-dichlorophenoxyacetic acid (2,4-D) by the presence of Fe(III) (hydr) oxides (Wu et al., 2010). V(V) reductase activity was membrane-associated as well as coupled the oxidation of NADH to the reduction of V(V), thus these mentioned species might also be conducive to V(V) reduction.

In another aspect, electrochemically active bacteria were also involved. The dramatically increasing Enterobacter of Proteobacteria could transfer electrons directly to other acceptors including solid electrode (Rezaei et al., 2009). The present Dysgonomonas was proved to produce electricity in MFCs (Liu et al., 2012). These species could be responsible for the bioelectricity generation in the proposed system.

There were also lots of fermentative microorganisms found in the MFCs with acetate though acetate is a non-fermentative substrate. The enriched Sedimentibacter could ferment several amino acids but could not use gelatin or sugars (Takii et al., 2007). Spirochaeta with the main characteristic of fermenting carbohydrates into simple organic acids was also enhanced (Sun et al., 2010). V(V) reductase activity was membrane-associated as well as coupled the oxidation of NADH to the reduction of V(V), thus these mentioned species might also be conducive to V(V) reduction.

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metabolites and they interacted with mentioned species together to facilitate V(V) reduction and energy recovery.

4. Conclusions

Acetate supported highest maximum power density of 589.1 mW/m², with highest V(V) removal efficiency of 77.6% during 12 h operation in MFCs, compared with glucose, citrate and soluble starch. Operating factors as initial V(V) and COD concentrations, conductivities and external resistances affected the performance of MFCs in aspects of V(V) reduction and power outputs. High-throughput 16S rRNA gene pyrosequencing analysis implied the accumulation of Enterobacter with the capabilities of V(V) reduction, electrochemical activity and fermentation in the MFCs. Other functional species as Pseudomonas, Spirochaeta, Sedimentibacter and Dysgonomonas were also enriched.

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