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Simultaneous arsenic(III) anaerobic oxidation with bioelectricity generation in microbial fuel cells

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ABSTRACT

Arsenic(As) is one of the most toxic elements commonly found in water and groundwater. Due to the limitation of anaerobic oxidation As(III) by traditional anaerobic treatment method, microbial fuel cells (MFCs) were applied to As(III) oxidation. A double chamber MFC with As(III) and sodium acetate(CH_3COONa) as electron donors was constructed, the transformation and migration of As(III) in the anode chamber under the stimulation of micro-voltage and the evolution of microorganisms were monitored. The results indicated that simultaneous As(III) anaerobic oxidation with bioelectricity generation were achieved only when CH_3COONa were required. The As(III) removal efficiency was 80% while the removal efficiency of traditional anaerobic treatment method was only 51% after a cycle of bioelectricity generation. The addition of As(III) with certain concentration could promote the power outputs of MFCs with itself being oxidized to less toxic As(V). Moreover, it was found that the migration of total As (including As(III) and As(V)) was from liquid phase to solid phase, and were enriched in sludge. It was also come to a conclusion that the activity of *Acinetobacter* and *Pseudomonas* with arsenic resistance was enhanced in the environment of long-term arsenic pollution by high throughput sequencing technology. This study offered a potential attractive method for remediation of arsenic-polluted groundwater.

Keywords: As(III) oxidation; Bioelectricity generation; Microbial community; Microbial fuel cells

1. Introduction

Arsenic is a common component of the earth's crust [1]. Its compounds are widely distributed in soil, sediments, water bodies and groundwater. Generally speaking, arsenic in natural minerals is insoluble. However, human activities, microbial degradation and chemical reactions may cause the weathering, migration and release of arsenic from insoluble phases into pore water, which will lead to arsenic pollution in groundwater, surface water and soil [2]. Arsenic pollution in water bodies has caused serious concern in environmental research. It is a well-known carcinogen, and its toxicity depends on its oxidation state. Inorganic arsenates including As(III) and As(V) are the main forms in water, and the former is more toxic [3]. As(III) dominates in the reducing environment, while As(V) dominates under oxidizing conditions. As(V) has less fluidity than As(III), adsorption fixation of As(V) is easier to be affected by iron or aluminum oxides and hydroxides in water [4]. Methods to remove dissolved inorganic arsenic include chemical oxidation [5] adsorption method [6], Fe(III) precipitation method [7], and microbiological law [8] etc. The combination of oxidation and adsorption has always been regarded

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as a remediation technology that can efficiently remove inorganic arsenic. The highly toxic As(III) is oxidized to As(V) by the oxidant firstly, and then the As(V) is trapped by adsorption to achieve the detoxification and removal of arsenic. Although As(III) can be efficiently oxidized by strong oxidants such as chlorine dioxide, sodium hypochlorite, potassium permanganate [9], the addition of these chemical reagents will increase the cost, and the reduction products may cause secondary pollution. In contrast, microorganisms are conducive to arsenic oxidase to catalyze the oxidation of As(III), which is a cost-effective and environmentally friendly treatment technology.

Activated sludge process is widely applied with dual functions of biological oxidation and adsorption. It can but only oxidize the highly toxic As(III) to the less toxic As(V) through microbial catalysis to achieve the detoxification of As, but also adsorb As(III) and As(V). Studies have found that arsenic-oxidizing microorganisms include As(III)-oxidizing bacteria (AOB), which can oxidize As(III) to As(V) under aerobic conditions using oxygen as an electron acceptor [10]. For anaerobic or anoxic groundwater environments, microorganisms use nitrate, nitrite and chloride as electron acceptors to complete the redox cycle of inorganic arsenic [11]. However, the biological oxidation capacity of activated sludge is controlled by the activity of microorganisms, that is, the metabolic growth of microorganisms affects the oxidation rate of As(III), in addition that As(III) itself has an inhibitory effect on the growth of microbes. During the biological oxidation of As(III) to As(V), it is very important that microorganism obtain energy to increase the efficiency of oxidation. There was a long history that electrochemistry can promote microbial metabolism [12]. Electrical stimulation can accelerate the transfer of extracellular electrons, promote biological processes, and improve processing efficiency [13], so it can break the bottleneck of low efficiency of As(III) anaerobic biological conversion to As(V).

Anaerobic oxidation of As(III) to As(V) can be achieved by using a polarized (+497 mV vs. SHE) graphite anode serving as terminal electron acceptor in the microbial metabolism firstly [14]. The proposed bioelectrochemical oxidation process would make it possible to provide As(III)-oxidizing microorganisms with a virtually unlimited, low-cost and low-maintenance electron acceptor as well as with a physical support for microbial attachment theoretically.

Microbial fuel cell (MFC) is an attracting increasing attention as it is capable of simultaneous pollutants removals and bioelectricity generation, offering an economic pathway to sustainable energy utilization and environmental protection [15]. Specific redox sensitive metal elements have been successfully handled in the anode chamber of MFCs with the combined effects of chemical and microbial oxidation [16]. Spontaneous anaerobic oxidation of As(III) in MFCs was realized [17]. When the initial concentration of As(III) in the anolyte is 200 ug/L, As(III) is completely oxidized to As(V) with low toxicity and low fluidity after 7 d, and the total organic carbon removal efficiency was 84%, which was the maximum 752.6 ± 17 mW/m^2 power density. It was more practical as spontaneous As(III) oxidation took place without extra energy consumption in the MFCs. However, to the best of the authors' knowledge, it is not clear that the relationship between As(III) oxidation and bioelectricity generation, so as to reveal the contribution to bioelectricity generation from anaerobic oxidation of As(III) to As(V). Moreover, the migration and distribution of arsenic in the anode chamber need to make clear, and the change process of microbial growth under the background of arsenic pollution are still unclear.

Therefore, this study was to: (1) investigate As(III) anaerobic oxidation spontaneously with bioelectricity generation; (2) establish the relationship between As(III) oxidation and bioelectricity generation; (3) discuss variation and distribution of arsenic in the anode chamber; (4) reveal the evolution of microbial community under the background of arsenic pollution. The main objective was to provide a potential attractive method for remediation of arsenic-polluted groundwater.

2. Materials and methods

2.1. MFC configuration and operations

Double-chamber MFCs as reported by the study of Guo et al. [18] in our previous study were built with anodes and cathodes compartments (working volume of 400 mL each).

A self-made carbon bush (effective volume of 80 mL) as the electrodes was used. Anode and cathode was electrically connected through an external copper circuit under a 1,000 Ω external resistance. A data acquisition card (DAQ Card, USB-4716, Yanhua Corp., China) was applied in the system to measure the output voltage and recorded data every 180 s throughout the whole experiment.

The catholyte of the cathode chamber used $K_3Fe(CN)_6$ (16.462 g/L)as the electron acceptors, and its pH value was around 7.0 and maintained by phosphate buffer solution (2.883 g/L KH_2PO_4, 6.571 g/L K_2HPO_4). The anolyte was simulated wastewater which contained the following components: 0.13 g/L KCl, 0.31 g/L NH_4Cl, phosphate buffer solution (2.883 g/L KH_2PO_4, 6.571 g/L K_2HPO_4), 12.50 mL/L microelement solution and 5.0 mL/L nutrient solution [18]. 0.641 g/L CH_3COONa (650.0 mg/L COD) chemical oxygen demand which was acted carbon source for anode microorganisms was added. The initial As(III) was added into the anolyte in the form of sodium arsenite ($NaAsO_2$) (0.0 mg/L As(III), 10.0 mg/L As(III), 20.0 mg/L As(III), 30.0 mg/L As(III), 40.0 mg/L As(III)). Inoculated anaerobic sludge was added to the chamber of all MFCs. The microorganisms of anode was from anaerobic sludge which were domesticated for 3 months with respective anolyte. Suspended solids and volatile suspended solids of the anaerobic sludge was 500.0 and 2,000.0 mg/L respectively.

Four MFCs as shown in the Table 1 with the same configuration as the proposed were marked with MFC-C, MFC-As and MFC-C-As and MFC-C-As-open. Compared the MFC-C-As, all running conditions were the same except that the wire was not connected in the MFC-C-As-open (It could be regarded as anaerobic wastewater treatment). These mixture were purged with argon gas (Ar) to decrease dissolved oxygen (DO) concentration of anode chamber. The initial pH of the anolyte was 7.0 by phosphate buffer solution. At the end of the bioelectricity generation cycle was defined as the voltage output decreased to about 50.0 mV. As(III) and As(V) concentrations in the anolyte

Table 1
Experimental operation conditions in the MFCs

Reactors No.	Sludge concentration (mg VS/L)	CH_3COONa concentration (mg/L)	As(III) concentration (mg/L)
MFC-C	500	650	0
MFC-As	500	0	20.0
MFC-C-As	500	650	20.0
MFC-C-As-open	500	650	20.0

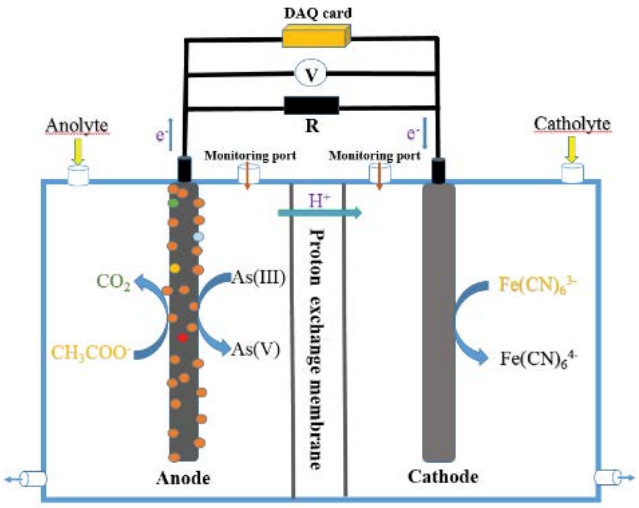

Fig. 1. Schematic of the MFCs.

were measured during a whole electricity generation cycle. Sludge samples were taken from the anode biofilm that had been running for 12 stable power generation cycles.

2.2. Analytical methods and calculations

2.2.1. Bioelectrochemical measurements

The output voltage (U, mV) was recorded using a multimeter data acquisition unit (PCI1713). The power density (P, mW/m^3) was obtained using $P = IU$, where I is current density (mA/m^3), U is the voltage (mV), A is the carbon bush volume (m^3). The potential of cathode and anode was measured by using Ag/AgCl electrode (+0.195 V vs. SHE) as reference electrode.

2.2.2. Chemical analyses

COD concentration of anolyte were measured according to standard methods during a whole bioelectricity generation cycle. Total As determination was quantitatively investigated according to the method published [19]. Total As in MFCs was divided into three parts: the part remaining in the solution ($[As]_{solution}$), the part removed by sludge and suspend sludge ($[As]_{flocs}$), and the part retained on the electrodes ($[As]_{electrodes}$). The initial total As concentration was denoted as $[As]_{initial}$. One part of the sample used for the As distribution analysis was filtered through a 0.45 μm membrane. The total As concentration in the filtration was denoted as $[As]_{solution}$. The other part of the sample for the As distribution analysis was performed by treating the sample (1 mL) with reducing agents (i.e., 1 mL HCl 37% and 1 mL of 5% ascorbic acid and potassium iodide solution, reaction time 30 min). Separation was performed in a Hamilton PRP-X100 anion-exchange column (250 mm × 4.1 mm, 10 μm) with $NH_4H_2PO_4$ as mobile phase (pH = 5.0) at the flow rate of 1.0 mL/min. Details of the As speciation method were described [18].

The total As concentration in the acidified solution was denoted as ($[As]_{solution+flocs}$). According to the above definitions, $[As]_{flocs}$ and $[As]_{electrodes}$ can be obtained by Eqs. (1) and (2):

$$[As]_{flocs} = [As]_{solution+flocs} - [As]_{solution} \quad (1)$$

$$[As]_{electrodes} = [As]_{initial} - [As]_{solution+flocs} \quad (2)$$

For the As(III) determination, samples from the anolyte were buffered to pH 5.0 by addition of acetate buffer. As(V) was calculated as the difference between total As and As(III).

2.2.3. Microbial community analysis

DNA samples was obtained from choosing the biofilm of anode of the six MFCs microbial. Small pieces of anodic carbon bush were trimmed and combined for DNA extraction. The total genomic DNA was extracted by using a soil DNA kit (OMEGA, USA) and amplified by specific primers (341F:CCCTACACGACGCTCTTCC GATCTGCCTACGGGNGGCWGCAG; 805R:GACTGGAG T T C C T T G G C A C C C G A G A A T T C C A G A C TACHVGGGTATCTAATCC) to target the hypervariable V3-V4 regions of the bacterial 16s rRNA gene [20]. Barcodes were modified in the 3′ terminus of the forward primer for sample multiplexing. For sequencing, libraries were normalized and pooled to MiSeq (Illumina, USA). Polymerase chain reaction (PCR) amplifications were performed in a 30 μL reaction mixture, which contained 1 μL of each primer (10 μmol/L), 20 ng of genomic DNA, 15 μL of 2 × Taq Master Mix and was added with nuclease free water to 30 μL. PCR cycling was conducted according to the following thermal conditions: an initial denaturing step at 95°C for 3 min and 5 cycles of denaturing at 94°C for 20 s; annealing at 55°C for 20 s and extension at 72°C for 30 s. For the last cycling program, the final extension step was held at 10°C. The PCR products were purified with an OMEGA gel extraction kit and quantified using a Qubit 3.0 fluorometer (Life Technologies, China). The Illumina MiSeq was applied for pooling and sequencing of the PCR products with equal DNA content for each sample.

3. Results and discussions

3.1. As(III) removal

The As(III) removal performance of MFCs was shown in Fig. 2a. The concentration of As(III) in MFC-As decreased to 18.0 mg/ L in 2 d, and then remained unchanged at 17.8 mg/L. The most likely cause is that the reduced As(III) in the solution may be adsorbed by suspended sludge or electrode. The concentration of As(III) in MFC-C-As decreased slightly from 19.8 to 6.8 mg/L in 12 d, then continue to decrease significantly from 6.8 to 4.2 mg/L for 15 d, the As(III) removal efficiency was up to 80%. However, when the MFC was operated at open circuit potential (MFC-C-As-open), the concentration of As(III) decreased from 20.0 to 12.8 mg/L in 12 d, then decreased slowly to 9.8 mg/L and remained unchanged finally, the As(III) removal efficiency abruptly decreased to 51%. A significant removal of As(III) concentration occurred in the MFC-C-As compared with MFC-C-As-open, which indicated that the micro-voltage generated from MFC-C-As maybe had a stimulation on the microorganisms, so that it accelerated the As(III) removal rate.

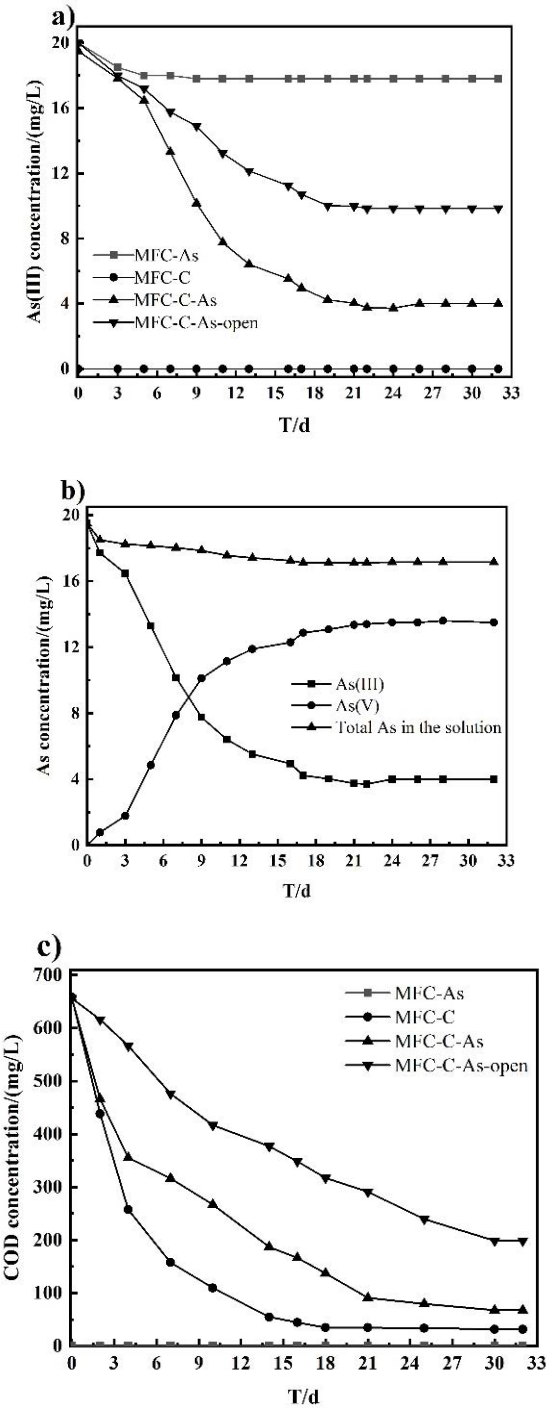

Fig. 2. Performance parameters (a) As(III) removal, (b) total As, As(III) and As(V) concentration, and (c) COD removal.

Significant As(III) removal was observed with corresponding generation of As(V) during the operation of MFC-C-As (Fig. 2b). After a power generation cycle, the As(III) concentration dropped from 20.0 to 4.2 mg/L with an increase in As(V) concentration from 0.0 to 13.2 mg/L. An almost linear decrease in the concentration of As(III) was observed in the early stage, which was mirrored by a stoichiometric increasing in the concentration of As(V). However, in the later stage of power generation cycle, the concentration of As(III) and As(V) both did not alter significantly. The experimental results showed that MFCs could oxidize As(III) to As(V) by anaerobic microorganisms without other energy input, it indicated that MFC could be considered to be an effective method to treat arsenic containing wastewater. A hybrid bioelectrochemical system coupling a MFC to a zero-valent iron (ZVI) technology was proposed for arsenic removal from aqueous solutions [19]. The arsenic removal efficiency in the MFC-ZVI hybrid process is much higher than that in the ZVI process, because the electric field produced by the MFC induces an extra electroadsorption force and improves the efficiency of arsenic removal on the electrodes in the MFC-ZVI process. The concentration of total arsenic (i.e., As = As(III) + As(V)) in the anolyte remained nearly constant throughout the tests except that about 2.0 mg/L total arsenic was adsorbed by sludge or electrode in the solution. Similar results were observed in previous studies where no significant arsenic adsorption was observed by using other virgin carbon materials as activated carbon [21–23], nanotubes [24].

COD removal in the anolyte is observed in Fig. 2c. After a power generation cycle of 32 d, the COD concentration of anolyte in the MFC-As was zero, and there were hardly any changes. The COD concentration of MFC-C decreased from 656.8 to 257.3 mg/L rapidly in 4 d. After 4 d, the decreasing rate slowed down, and finally stabilized at about 31.2 mg/L. The COD concentration of MFC-C-As-open decreased slowly from 656 to 198 mg/L. Correspondingly, the concentration of COD decreased linearly from 657.2 to 355.4 mg/L in 4 d, and the concentration of COD continued to decrease after 4 d in MFC-C-As, the rate decreased significantly. Although CH_3COONa can act as an important and effective electron donor for oxidation reaction, the addition of As(III) with certain concentration could restrain the COD removal with itself being oxidized to less toxic As(V).

As we all know, electrons from oxidation of both As(III) and CH_3COONa were transferred to solid anode electrode

through electricigens for bioelectricity generation [25], they could be seen in Eqs. (3) and (4).

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad E_\theta = 0.187V \quad (3)$$

$$AsO_2^- + 2H_2O \rightarrow AsO_4^{3-} + 4H^+ + 2e^- \quad E_\theta = -0.56V \quad (4)$$

In addition to CH_3COONa, glucose as organic carbon sources is a common inorganic carbon source in the MFCs [26]. Since the electrons for electricity generation originated from electron donors [27], it is natural that electron donors were responsible in large part for releasing electrons. So what was its contribution to bioelectricity generation in the MFC-C-As?

3.2. Bioelectricity generation

Voltage outputs in a typical cycle were recorded and exhibited in Fig. 3a. It could be seen that the output voltage of MFC-As could be negligible, and the power generation

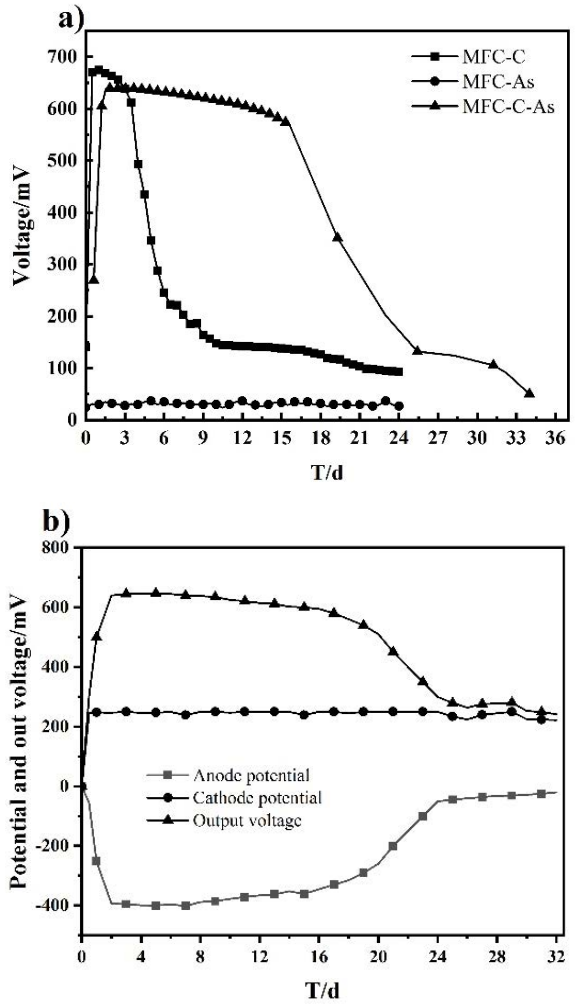

Fig. 3. Power outputs of the MFCs (a) voltage outputs and (b) potential change in the MFC-C-As.

capacity of MFC-C-As was significantly improved due to the addition of CH_3COONa as carbon source. The output voltage increased rapidly from 20.0 to 645 mV in 0.5 d, and the high stable operation time is close to 15 d, then decreased rapidly after 16 d, and slowed down and tended to be stable after 24 d (60 mV). Although the output voltage of MFC-C increased rapidly to 650 mV in 0.5 d, the cycle of high voltage operation is only about 4 d. Compared with MFC-C-As, the power generation cycle was significantly shorter. It can be concluded that bioelectricity generation and As(III) removal could not be realized using As(III) as sole electron donor. Maybe As(III) is a recalcitrant compound and cannot be used as sole carbon source to directly provide energy to the microorganisms. Therefore CH_3COONa is necessary to support the growth of microorganisms. Higher bioelectricity was obtained in the MFC-C-As than MFC-C, indicating that As(III) with relatively lower valence is a reducing substance, even electrons from oxidation of both As(III) and CH_3COONa were transferred to solid anode electrode through electricigens for bioelectricity generation.

Electron balance was also calculated. There were two electron sources in the MFC-As-C, that is, CH_3COONa oxidation (1,561.5C) based on COD removal (from 657.2 to 60.4 mg/L) and As(III) oxidation (0.034C) based on As(V) generation (from 0.0 to 13.4 mg/L), while about 170.2C of electrons based on were transferred to external circuit for bioelectricity generation during a whole the bioelectricity generation circle, indicating that the contribution of electrons from As(III) oxidation to the current is very limited, however, most electrochemically active bacteria could still survive with As(III) addition and function well in the MFC-As-C. Additionally by using organic matter glucose and As(III) as the electron donor, As(III) removal efficiency was similar to that obtained by the poised bio-anode with the advantage of a spontaneous process reported by Pous et al. [14]. Nevertheless, due to the general lack of organic matter in groundwater or surface water, glucose or another organic carbon source should be dosed carefully, in order not to create an additional groundwater contamination.

It is widely known that the output voltage depends on the relative difference between cathode potential and anode potential in the MFCs. Fig. 3b displays the trend change of electrode potential and output voltage of MFC-C-As. From the experimental results, it stated clearly that the cathode potential and anode potential were very different in the power generation cycle, the fluctuation of cathode potential was negligible, and it could maintain at –250 mV so that there are enough electrons to supply it. The anode potential declined to –393 mV quickly within 2 d, reached the maximum –401 mV on the 4th day, then maintained the low potential operation, slowly increased to –315 mV after 20 d. After 14 d operation it decreased to –50 mV. It was declared that a power generation cycle ended. The change of output voltage is just opposite to the change of the anode potential, indicating that the fluctuation of output voltage is mainly regulated by anode potential.

The output voltage of MFC-C-As was closely related to the oxidation process of carbon source (including CH_3COONa and As(III)). Combined with the above experimental results of As(III) removal, CH_3COONa removal and bioelectricity generation of MFC-C-As, the anode

potential quickly moved to a higher level within 2 d, which was mainly from the oxidation of CH_3COONa. From the 4th day, the oxidation efficiency of As(III) increased, and more electrons were released. The anode potential of MFC-C-As was extended at high position and lasted until about 20 d until As(III) and CH_3COONa were exhausted. The CH_3COONa concentration was not enough to support the growth of microorganisms, so that the anode potential of MFC-C-As dropped sharply, and a power generation cycle came to an end. Similar phenomenon had also been observed when responding pollutants were treated, such as V(V)/V(IV) and SO_4^{2-}/S^{2-} [28,29]. This indicated that the addition of As(III) with certain concentration could promote the power outputs of MFCs with itself being oxidized to less toxic As(V), however, resistant As(III) oxidizing bacteria cannot survive for a long time without additional carbon source.

3.3. Relationship between As(III) removal and bioelectricity generation in the MFCs

As shown in Fig. 4, the NO_3^-–N removal rate and the maximum power density changed simultaneously at different initial As(III) concentrations in the MFC-C-As.

When the As(III) concentrations was 20.0 mg/L, the maximum power density and the maximum As(III) removal rate were obtained. They were 7300.8 mW/m^3 and 0.36 mg/(L/h) respectively. These results indicated that the bioelectricity generation ability correlates well with the As(III) removal rate.

Generally speaking, the bioelectricity generation performance of MFCs is mainly determined by the electron release rate of the anode and the ability of the electron acceptor of the cathode. The latter is affected by the environmental conditions of the cathode chamber. From the previous experimental results, the cathode conditions of MFCs are almost the same at different As(III) concentrations, so that there is almost no fluctuation of the cathode potential. The former is mainly related to the environmental conditions of the anode chamber. Except for the different concentrations of As(III) in the cathode chamber, other conditions including COD are the same, but the anode potential has changed significantly. This showed that the change of As(III) concentration affects the power generation performance of MFCs. As the concentration of As(III) increased, more electrons from oxidation of both As(III) and CH_3COONa were transferred to solid anode electrode through electricigens for bioelectricity generation [25]. However, the bioelectricity generation was reduced because of too much As(III) adding. It indicated that the addition of As(III) with certain concentration could promote the power outputs of MFCs with itself being oxidized to less toxic As(V), however, As(III) with high concentrations could inhibit the activity of electrochemically active bacteria and cut down the output of electrical energy.

3.4. Variation and distribution of arsenic in the MFCs

Fig. 5 showed the transformation of the total arsenic (including As(III) and As(V)) in the MFC-C-As and MFC-C-As-open after a power generation cycle. The total arsenic was present in three states: in solution, on the electrodes and in the flocs. It was found that the total arsenic of the effluent was lower than 20.0 mg/L of the total arsenic of the influent water, which indicated that the total arsenic was reduced after anaerobic oxidation. In the MFC-C-As-open reactor, 91% of the total arsenic remained in the solution, 6% of the total arsenic was detected in flocs including the sludge and suspended sludge, and the remaining 3% of the total arsenic was on electrodes. Correspondingly, 87% of the total arsenic reactor remained in the solution, 11% of the arsenic was detected in flocs, and the remaining 2% of the arsenic was on electrodes in the MFC-C-As.

According to the As distribution, the content of soluble arsenic in MFC-C-As was reduced, which promoted the transfer of arsenic to the solid phase, which may be related to the micro-voltage generated by the system. The micro-voltage from the MFC-C-As could strengthen the oxidation of As(III) to As(V), so that the migration of As was weakened and it was easy to migrate to the solid

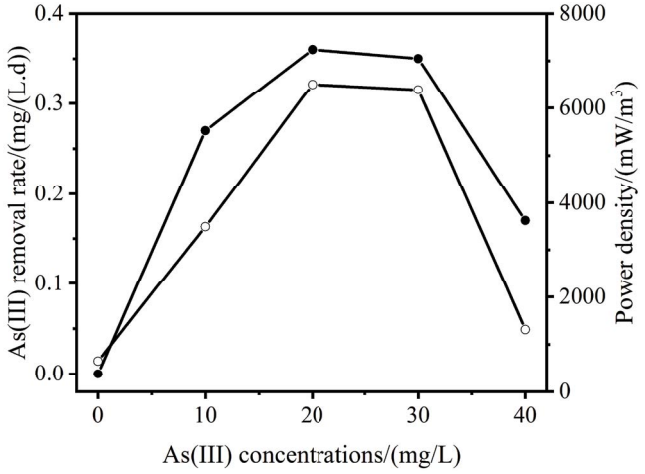

Fig. 4. Relationship between the maximum power density and the As(III) removal rate.

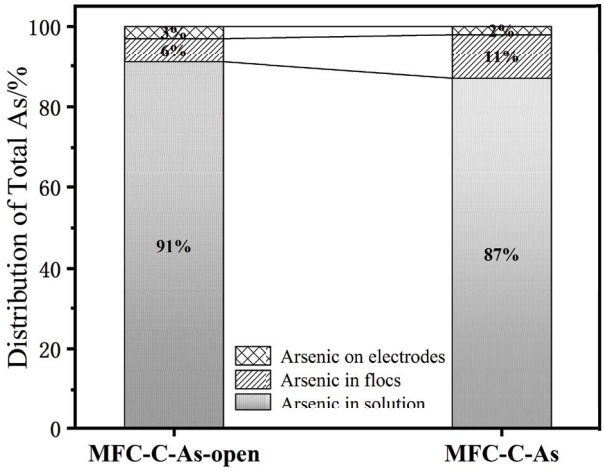

Fig. 5. Distribution of total arsenic in the MFC-C-As and MFC-C-As-open.

phase. Ji et al. [30] combined sulfate reduction with BES to intensify the degradation of roxarsone. Under the combined action of electrical stimulation and sulfate reduction, roxarsone was completely degraded within 13–22 d, while roxarsone was completely degraded within 13–22 d by traditional anaerobic digestion. It was further discovered that roxarsone in the system was degraded to inorganic arsenic, and 75.0%–83.5% of arsenic could be effectively fixed, which was significantly higher than traditional anaerobic digestion (41.2%–57.3%). BESs could improve the efficiency of arsenic fixation. The fundamental reason was to promote the precipitation of arsenic. Adsorption after oxidation was an effective strategy to completely solve the problem of arsenic pollution [31]. Although the application of micro-voltage had an effect on the oxidation efficiency of As(III) and the fixation of As [14], the MFC system is more meaningful, because there is no additional energy consumption during the oxidation of As(III), even there is an output of bioelectricity output.

3.5. Microbial community

Microorganisms are the core element of microbial fuel cells, because the anaerobic biological reaction in the anode chamber is the result of the action of various microorganisms. The structure of the microbial community changes due to the operating time and operating factors of the reactor. In order to understand the difference of the microbial community in the anode chamber and the change in composition over time, the original inoculated sludge (Raw sludge), the biofilm attached to the electrode material in the anode chamber (MFC) after the end of the 12 stable power generation cycle operation experiment (MFC-C-As) and the biofilm (MFC-C-As-open) attached to the electrode material in the anode chamber under the same conditions under the same conditions, classification and analysis are performed at the sequence level at the phylum and genus level.

The predominant archaeal phylum detected in all the MFCs were *Proteobacteria* and *Bacteroidetes* (Fig. 6a), and a small amount of *Planctomycetes*, *Chloroflexi*, *Firmicutes* and *Actinobacteria*. *Proteobacteria* accounts for 34.98%–77.31%, and *Bacteroidetes* accounts for 7.2%–16.65%. *Gammaproteobacteria*, *Bacteroides*, and *Betaproteobacteria* are common flora in MFCs [32]. Most of the bioelectricity-producing bacteria reported are distributed in *Proteobacteria* including *Shewanella* [33]. It is the model strain for the research of electric-producing bacteria. Compared with the anode electrode membrane of raw sludge (without adding As(III)), the proportion of

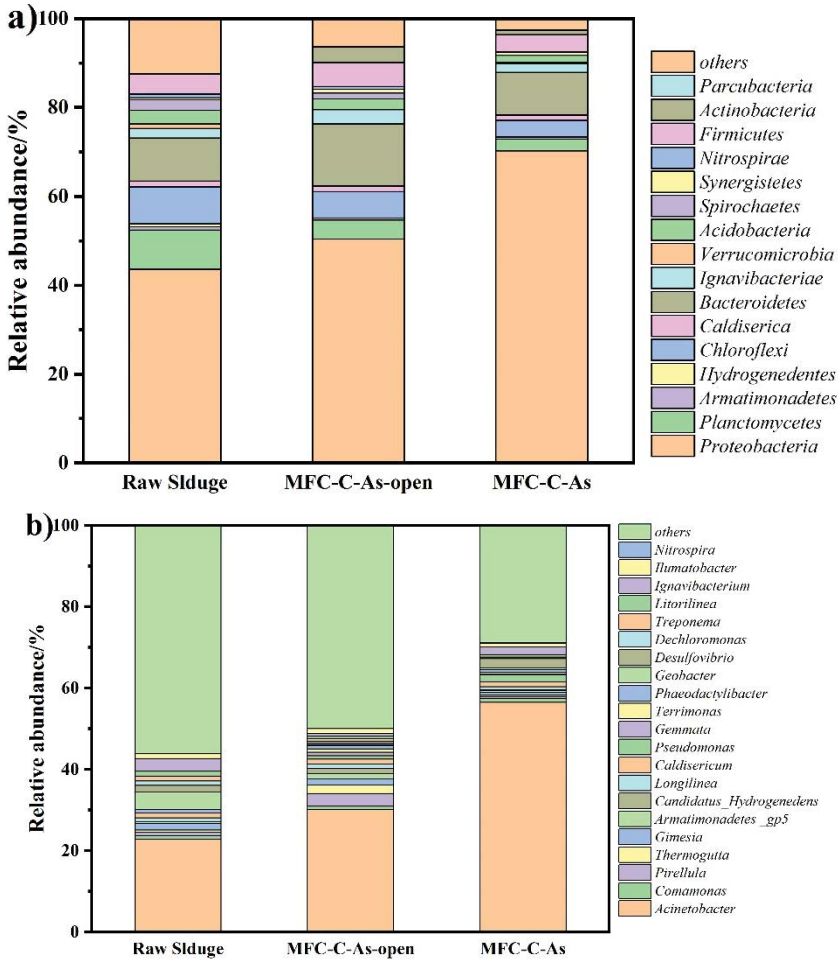

Fig. 6. Bacterial community compositions obtained from the anode biofilms at: (a) phylum level and (b) genus level.

Proteobacteria and *Bacteroidetes* with adding 20.0 mg/L of As(III) concentration increased, however, the proportion of *Proteobacteria* decreased in the MFC-C-As-open, which indicated the growth of *Proteobacteria* could be inhibited without micro-voltage. Azarbad et al. [34] found that the bacterial community in arsenic-contaminated soil was significantly different from the uncontaminated soil, and *Proteobacteria* was more resistant to arsenic. Oremland et al. [35] found that MLHE-1 whose 16S rRNA sequence is placed in the *α- Proteobacteria* of haloalkaliphilic *Ectothiorhodospira*, that is, *Proteobacteria* played an important role in degradation and transformation As(III) about anaerobic oxidation and facultative arsenite oxidation of arsenite in fresh lake water. Zhang et al. [36] isolated a new type of chemical autotrophic oxidizing bacteria SY from contaminated paddy soil and found that the oxidation of As(III) was the detoxification mechanism of the bacteria.

More than 24 genotypes of genus including *Acinetobacter*, *Geobacter*, *Ignavibacterium*, *Comamonas*, *Pirella*, *Thermogutta* and *Desulfovibrio* were discovered on the anode electrode membrane while remarkable change happened. *Acinetobacter* increased significantly that suggested structures of the bacteria community had evolved as adapting to the new conditions during the operation (Fig. 6b).

The top three bacterial genera in the list are: *Acinetobacter*, *Geobacter*, and *Ignavibacterium* accounting for 22.86%, 4.34%, and 3.18%, respectively in the raw sludge. The abundance of *Acinetobacter*, *Pirella* and *Thermogutta* accounting for 30.01%, 3.16% and 2.11% were the top three in the MFC-C-As-open. The top three proportion of *Acinetobacter*, *Desulfovibrio*, and *Ignavibacterium* were 56.42%, 2.31% and 1.98% in the MFC-C-As.

The experimental results showed that *Acinetobacter* was dominant in all samples tested. This was consistent with the findings of Li et al. [37]. Li et al. [38] has found the main dominant bacteria in high arsenic groundwater is *Acinetobacter*. *Acinetobacter* was also a major genus in high arsenic aquifers and other high arsenic aquifers in the mountainous region of Bangladesh and northwestern China. These microorganisms contained many arsenic-resistant strains, and they could participate in the arsenic geochemical cycle. Some *Acinetobacter* can oxidize and reduce arsenic. *Ignavibacterium* also appears in the community [39].

This bacterium was facultatively anaerobic and can be fermented with organic matter. It was originally found in deep-sea hot springs. *Desulfovibrio* in the community was a typical sulfate-reducing bacterium that was resistant to arsenic [40]. Macy et al. [41] isolated *Desulfomicrobium* strain sp. from in situ sediments, which contains arsenic resistance gene. In addition, the relative abundance of *Pseudomonas* was 1.84% in MFC-C-As anode chamber samples and only 0% and 0.84% in control group samples. Heterotrophic arsenite oxidizing microorganisms were isolated from [42] *Pseudomonas* (*Pseudomonas* sp.hn-1 and *Pseudomonas* sp.hn-2) and used CH_3COONa as the source of cell material and energy in anode chamber, As(III) as an electronic donor, then As(III) was oxidized to As(V).

In general, the above experimental results showed that that the composition of the anode microbial community was relatively stable. Mixed cultures with the higher microbial diversity, great adaption, self-evolution abilities were used in this paper [43], while As(III) was oxidized more quickly compared with pure cultures in anaerobic environment [44]. The mixed cultures contained both electric-producing bacteria and arsenic oxidation bacteria. However, the interrelationships between the various bacteria groups are very complex. Especially, the addition of As(III) changed the abundance and structure of microorganisms, which indicated that arsenic was a type of stress factor and selective pressure. It had an impact on the physiological metabolic process, survival, adaptation to the environment and the ecological functions of anode microorganisms. In these experiments, arsenic oxidation microorganisms and electric-producing microorganisms intersected with each other in the MFC-C-As, so that the working mechanism is not clear so far and required further research.

4. Conclusions

The MFC-C-As had the dual properties of generating bioelectricity and promoting spontaneous anaerobic oxidation of As(III). When the initial As(III) concentration and COD concentration in the anolyte are 20.0 mg/L and about 650 mg/L, the output voltage reached 639.7 mV, the maximum power density and the maximum As(III) removal rate were 7300.8 mW/m^3 and 0.36 mg/(L/h) respectively, indicating that the bioelectricity generation ability correlates well with the As(III) removal rate. However, more than 20.0 mg/L As(III) could inhibit the activity of electrochemically active bacteria and the ability of generating bioelectricity. Moreover, the total As(including As(III) and As(V)) were enriched in flocs maybe owing to the micro-voltage from the MFC-C-As. High-throughput sequencing analysis indicated that the predominant phylum was *Proteobacteria* and *Bacteroidetes*, and the predominant genus was *Acinetobacter* and *Desulfovibrio*. Arsenic-resistant bacteria and electrochemically active bacteria interacted together and they were responsible for As(III) oxidation and bioelectricity generation.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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