

Behavior of Copper, Nickel, Cadmium and Mercury Ions in Anode Chamber of Microbial Fuel Cells

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Microbial fuel cell (MFC) technology is widely used in wastewater treatment, and heavy metal ions play an important role in MFCs. In this paper, we studied the effect of heavy metal ions (copper, nickel, cadmium and mercury) on anode, mainly in voltage output and the recovery after feeding with high concentration of those heavy metal ions. It was concluded that anolyte containing low concentration of Cu^{2+} and Ni^{2+} can improve the performance of MFCs, but when the concentration becomes higher, the voltage outputs decreased, the minimum toxic concentration of Cu^{2+} is $303 \mu\text{mol/L}$ and Ni^{2+} 's is $600 \mu\text{mol/L}$. As for Cd^{2+} and Hg^{2+} , the voltage outputs decreased once these ions were added into the MFCs. After replacing the anodic solution with a refresh solution that containing no heavy metal ions, the MFCs would recover in several hours (Cu^{2+} , Ni^{2+} and Cd^{2+} in 24 h, Hg^{2+} in 30 min).

Keywords: Microbial fuel cells; Voltage output; Anode; Metal ions

1. INTRODUCTION

In recent years, Microbial fuel cells (MFCs) have become a cutting-edge topic of many environmental research efforts, since it can be used to treat the wastes and to harvest energy and products simultaneously [1], like copper [2-4], nickel [5], cadmium [6,7]. With the advantages of clean, high efficiency and the mild reaction condition, MFCs are widely used in wastewater treatment. However as we all know, heavy metal ions exist in wastewater treatment widely and variously and they play an important role in each part of MFCs especially in anode. So the behavior of heavy metal ions become a research subject that can not be neglect.

In general, Metal ions in MFCs can be reduced by metal-reducing enzymes or dissimilatory metal reduction microorganisms (DMRM). Therefore, the metal ions can be removed or recovered

through MFCs. In other words, metal ions can be electron acceptor in MFCs. Yan Li. established a self-sustained hybrid bioelectrochemical system consisting of microbial fuel cell (MFC) and microbial electrolysis cell (MEC) to reduce multiple metals simultaneously by utilizing different reaction potentials, like lead and nickel[5]. N.Touach. structured a cathode based on $\text{MnO}_2/\text{CNT}/\text{PTFE}$ to remove heavy metals(Cu, Zn, Cd, Sb, Pb, Mn and Fe) in MFCs [8]. Recovery of copper from wastewater containing ammonia-copper (II) complexes via the cathodic reduction of $[\text{Cu}(\text{NH}_3)_4]^{2+}$ using a dual chamber MFC was investigated by Zhang [9]. Wang removed Hg^{2+} from the wastewater coupled with power generation [10].

Behaviors of heavy metal ions in MFCs are complex. Each MFC including biocatalysts, electrolyte, membranes and electrodes are influenced by the sophisticated behavior and transformation of heavy metal ions [11]. Metal ions can promote the activity of the biocatalysts by serving as mineral elements or electron transfer mediators [12,13]. As for electrolyte, heavy metal ions have effects on electrolyte conductivity and internal resistance [14,15]. It may result in negative effects for long term operation of the MFCs because of the precipitation of metal ions on the membrane [16]. Some heavy metal ions can be involved in the cathodic process as electron acceptors, which directly affect the performance of MFCs [17], and it can indirectly participate in the anodic chemical reactions on the other hand. Furthermore, the health of microorganisms, in other words, the biotoxicity of heavy metal ions can influence the performance of MFCs [18].

It was known that heavy metal ions are toxic to the microorganisms or enzymes, including Cu^{2+} , Hg^{2+} and Cd^{2+} . [19], which may lead to negative effect on the performance of MFCs. Kim found that inhibition ratio of 46%, 28%, respectively in MFCs were observed in synthetic wastewater with addition of 1 mg/L Cd^{2+} and 1 mg/L Pb^{2+} mixture. While higher inhibition ratio of 76 % were observed in real wastewater with addition of 1 mg/L Cd^{2+} and Pb^{2+} mixture [20]. However, the MFCs also showed a certain degree of tolerance at lower concentrations of heavy metal ions. The bioelectrocatalytic performance was not influenced by the metal ions with a certain degree of concentrations for wastewater-derived electroactive microbial biofilms in MFCs [21]. Stein also found that higher overpotential and higher current density caused by a decreased concentration of the toxicant led to a higher sensitivity of the sensor [22]. Therefore, the immediate response to the toxicity of heavy metals in MFCs indicates that they can be applied to biomonitor.

In this study, we evaluated the behavior of copper, nickel, cadmium and mercury ions on their transformation and distribution in the anodes of MFCs, including the effect on voltage output, migration and distribution. As for biotoxicity, the minimum toxic concentration and recovery of MFCs are researched.

2. EXPERIMENTAL

2.1 MFC construction

Each MFC consisted of two cylindrical vitreous bottles (H-type reactor). The anodic and cathodic chambers were separated by a cation exchange membrane (CEM, 13 cm², CMI7000, Dupont,

USA). The membrane was pretreated by submerging in H_2SO_4 solution (1 mol/L) for 24 h, rinsing with DI water, and air drying. The experiment of different ions exist some difference. The electrode was made by graphite felt, the difference between batches are shown in Table. 1. Additionally, the anode was wrapped inside a nylon net (80 mesh, Jinlong Electronic Material Co. Ltd., China). Titanium wires (Sanxin Metal Co. Ltd., China, 1.0mm) were used to connect the two electrodes, and plastic screws were employed to secure the connection.

Table 1. The specifications of electrode in different batches.

Batches of ions	Specification of anode	Specification of cathodic
Copper	12 cm ² (3 cm×4 cm)	12 cm ² (3 cm×4 cm)
Nickel	4 cm ² (2 cm×2 cm)	4 cm ² (2 cm×2 cm)
Cadmium	Φ4 mm×14 cm	Φ4 mm×14 cm
Mercury	12 cm ² (3 cm×4 cm)	12 cm ² (3 cm×4 cm)

2.2 MFC inoculation and operation

Six reactors were constructed as specified above, and designated as FC (abiotic), MFC-0 (blank, no metal added), MFC-1, MFC-2, MFC-3 and MFC-4 (Fig. 1.) All 5 MFCs were inoculated with a mixed microbial culture containing 50 mL of MFC consortium previously isolated from the sludge of a wastewater treatment plant (name, location, China) growing on acetate. Anodic nutrient medium of 130 mL was then added to each MFC. This nutrient medium contained (per liter) 1.64g NaAc, 0.5g NH_4Cl , 0.1g $MgSO_4$, 0.08g $CaCl_2$, 0.1g KCl , 2.0g $NaCl$, 5.46g $Na_2HPO_4 \cdot 12H_2O$, 1.22g $NaH_2PO_4 \cdot 2H_2O$ and 1.0 mL trace elements [23] (pH=7.0). The mixture was purged with N_2 for 10-15 min to achieve the anaerobic condition [24]. The cathode solution (180 mL) contained 32.92g $K_3[Fe(CN)_6]$, 5.46g $Na_2HPO_4 \cdot 12H_2O$ and 1.22g $NaH_2PO_4 \cdot 2H_2O$ per liter (pH=7.0). The anode solutions were mixed continuously by placing the anode chambers on a six-magnetic stirrer plate (rotating speed of 200 rpm) [25](JJ6, Boke Test Equipment Institute, China). All MFCs were operated in batch mode under room temperature ($25 \pm 1^\circ C$).

After starting-up, polarization curves were tested to determinate the internal resistance, and chose the external resistance. Gradient increase of each metal ions were adding into anode of MFCs to investigate the effect on voltage output. Gradient increase were also adding to investigate the maximum tolerance concentration. MFCs were split to investigate the distribution and the species of metal ions in biofilm.

After each batch, sample the anode effluent to measure the concentration of the heavy metal ions. Two drops of (1+1) nitric acid were adding into the samples to keep the sample acidity. There were precipitation on the bottom of the sample bottles due to the exist of PIPES, but the precipitation did not contain elemental metal (depend on the result of EDS on the precipitation).



Figure 1. The reactors constructed for experiments were placed on a six-magnetic stirrer plate with a rotating speed of 200 rpm. Each MFC consisted of two cylindrical vitreous bottles (H-type reactor) with the volume of 180 mL. The anodic and cathodic chambers were separated by a cation exchange membrane (CEM, 13 cm², CMI7000, Dupont, USA). Both electrodes were made with graphite plates with a working area . Titanium wires were used to connect the two electrodes with an external resistance. All MFCs were operated in batch mode under room temperature (25±1 °C).

2.3 Analysis

Cell voltages were recorded every 5 min by a data acquisition system (Model 2701, Keithley, Cleveland, OH, USA). Polarization curves were constructed using power density data obtained with an electrochemical workstation (PARSTAT 2273 Princeton, USA). The optimal external resistance was selected based on the polarization curves. The power density was calculated according to the equation $P=V \times I/A$, where I is the current, V is the voltage and A is the area of an anode.

The effluent was sampled (10 mL) to measure the metal ion concentration through Atomic Absorption Spectrometry (novAA400, Analytic Jena AG, German). A drop of nitric acid solution (50% in water) was added into each sample to maintain the ionic form of metal. Coulombic efficiency (CE) was obtained by calculating the ratio of total recovered coulombs by integrating the current over time to the theoretical amount of coulombs that can be produced from acetate. Detailed information can be found in a previous report [26]. Removal efficiencies of the ion were calculated according to $(C_i - C_e) \times 100 / C_i$, where C_i and C_e are the ion concentrations in the influents and effluents of the MFCs, respectively.

3. RESULTS AND DISCUSSION

3.1 MFC start-up

After 10 days of inoculation and acclimatization, the voltage output of copper MFCs increased to 0.60-0.61 V, since the data of nickel are 30 days and 0.68-0.7 V, cadmium are 7 days and 0.46-0.47 V and mercury are 15 days and 0.5-0.55 V. Polarization curves were constructed. The maximum power density of copper MFCs was around 500 mW/m², corresponding to an internal resistance of about 500 Ω. Accordingly we selected an external resistance of 500 Ω for copper MFCs. The other batches are analysed in same way, and the electric parameters are shown in Table. 2. The voltage output was stable during the first 50 hrs for each cycle of power generation; therefore 48 hrs was selected as the optimal processing duration.

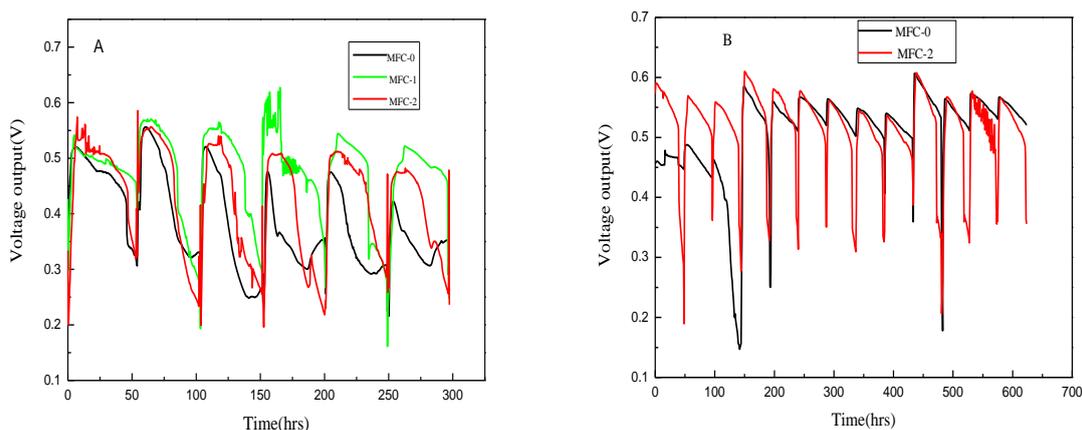
Table 2. Electric parameter of different batches

Ions	Acclimatization time(d)	Voltage output(v)	Maximum power density(mW/m ²)	Internal resistance(Ω)
Copper	10	0.60-0.61	500	500
Nickel	30	0.68-0.70	150-200	5000
Cadmium	7	0.46-0.47	700-750	5000
Mercury	15	0.50-0.55	800	500

3.2 Effect of heavy metal ion on voltage output of the MFCs.

3.2.1 Copper

The variation of voltage output was observed in Fig. 2A. The average voltage outputs of MFC-0 and MFCs with Cu²⁺ of both 43.3 and 86.7 μmol/L were 0.511, 0.534 and 0.553 V, respectively. The voltage outputs of MFCs with Cu²⁺ of both 43.3 and 86.7 μmol/L were higher than those of the blank (MFC-0), indicating that at these concentrations Cu²⁺ could improve the performance of MFCs.



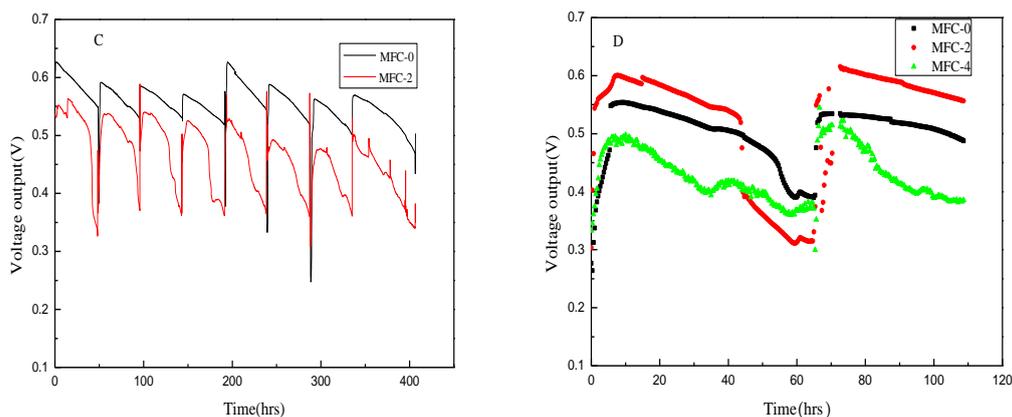


Figure 2. Variation of voltage output in MFCs: A) These reactors contained low concentrations of Cu^{2+} with $43.3 \mu\text{mol/L}$ in MFC-1 and $86.7 \mu\text{mol/L}$ in MFC-2; B) Cu^{2+} concentration gradually increased from 130 to $649 \mu\text{mol/L}$ with $43.3 \mu\text{mol/L}$ interval additions in MFC-2; C) Cu^{2+} concentration gradually increased from 779 to $1385 \mu\text{mol/L}$ by $86.7 \mu\text{mol/L}$ interval additions in MFC-2; D) At $1559 \mu\text{mol/L}$ Cu^{2+} , the anolyte in MFC-2 was replaced with fresh anolyte containing no Cu^{2+} . Cu^{2+} concentration of MFC-4 was at $1559 \mu\text{mol/L}$. MFC-0 was the no-copper control for all panels.

This result seemingly contradicted the generally accepted principle of low Cu^{2+} tolerance of microbes [27-29]. Further analysis suggested that the actual Cu^{2+} concentration remaining in the anodic solutions was much lower. For example, when $86.7 \mu\text{mol/L}$ Cu^{2+} was added to the anode chamber, only $2.65 \mu\text{mol/L}$ Cu^{2+} could be detected after the batch reactions. Therefore, these microbes were not affected at the added levels of copper ions.

As discussed above, low $[\text{Cu}^{2+}]$ can improve the performance of MFCs, while high $[\text{Cu}^{2+}]$ undoubtedly would be toxic to microbes. When Cu^{2+} was gradually added to anodic chambers of MFCs at $43.3 \mu\text{mol/L}$ intervals from Exp. 3 to Exp. 16, we obtained 13 batches of anode solutions with $[\text{Cu}^{2+}]$ ranging from 128.8 to $649.5 \mu\text{mol/L}$. Results showed that $303 \mu\text{mol/L}$ (resulting $[\text{Cu}^{2+}]$ after batch operation was $5.61 \mu\text{mol/L}$) or less added copper increased the voltage output in MFCs in comparison with the blank control (MFC-0); at concentrations higher than $347 \mu\text{mol/L}$, the voltage output of the blank was higher than that of MFCs with Cu^{2+} ; and when $[\text{Cu}^{2+}]$ was between 303 and $347 \mu\text{mol/L}$, the voltage outputs of MFC-0 and MFCs were about at the same level (Fig.2B). Therefore it's concluded that the MTC for these microbial fuel cells was 303 - $347 \mu\text{mol/L}$, which was higher than the MTCs of Cu^{2+} ($218.8 \mu\text{mol/L}$) reported by Pamukoglu and Kargi [30]. The difference might be because the buffer capacity of the anolyte contained 20 mmol/L phosphate salts. The dissolved copper concentration (accurately, it is exchangeable copper concentration) but total copper concentrations are directly toxic to microbes. In this research, part of Cu^{2+} was converted into copper phosphate and copper hydroxide because of phosphate salt and neutral pH conditions. When we examined 8 MFC batches supplemented with $[\text{Cu}^{2+}]$ of 779 to $1385 \mu\text{mol/L}$, we observed decreased voltage output as $[\text{Cu}^{2+}]$ increased. MFCs however could still work well with a maximum voltage above 0.4 V (Fig.2C).

The Coulombic efficiency (CE) of the no-copper MFC control was $37.62 \pm 7.8\%$, while the experimental MFCs with $86.7 \mu\text{mol/L Cu}^{2+}$ had CE values averaging $28.26 \pm 5.4\%$. The decrease of CE values could be best explained by the consumption of chemical oxygen demand of Sulfate Reducing Bacteria (SBR), and their generated sulfide ions reacting with Cu^{2+} causing precipitations. To balance the ionic concentrations, COD was continuously consumed to reduce sulfate to sulfide by SBR. Cu^{2+} could also affect Coulombic Efficiency in a similar manner as oxygen, but reduction to Cu^0 or Cu^+ can cause CE and the electric output to increase. Since COD in each MFC was 160 mmol/L , while Cu^{2+} was only 0.078 mmol/L ($\text{Cu}^{2+}/\text{COD}$ is 0.0004875), the influence of Cu^{2+} would be too small to be noticeable.

3.2.2 Nickel

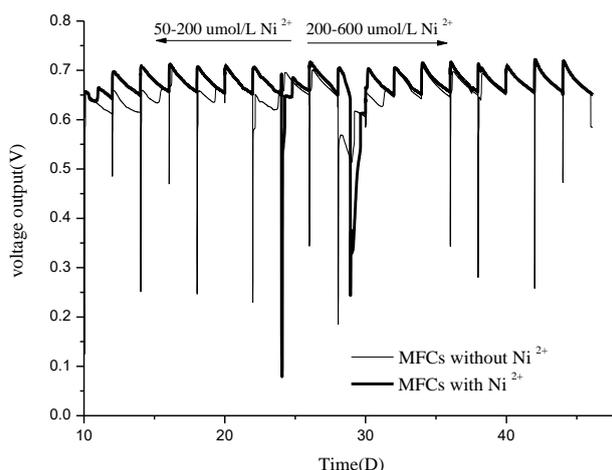


Figure 3. Voltage output of MFCs with Ni^{2+} concentration of $50\sim 600 \mu\text{mol/L}$ stepped by $50 \mu\text{mol/L}$.

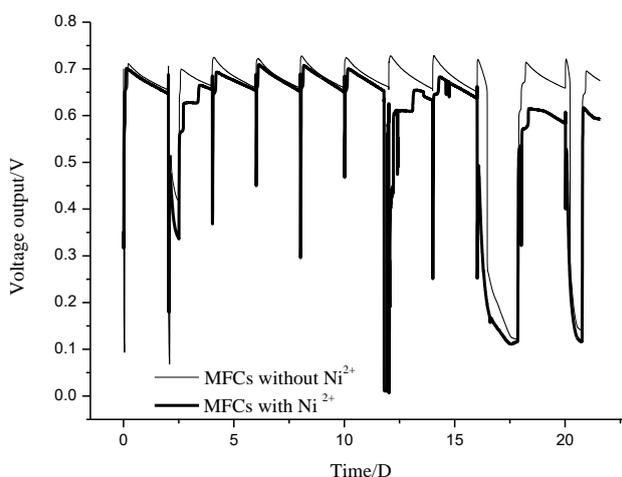


Figure 4. Voltage output of MFCs with different concentrations of Ni^{2+} ($600\sim 1000 \mu\text{mol/L}$, stepped by $100 \mu\text{mol/L}$)

The voltage outputs were showed in Fig. 3, the voltage outputs of first four batches were not stable because of the low and unstable temperature. In order to eliminate the effect of temperature, the

MFCs were playing on the electric water bath, and keep the temperature on $30 \pm 1^\circ\text{C}$. Subsequently, the voltage outputs of all batches of MFCs were above 0.6 V, higher than their control reactors. In the first several batches, that is the nickel concentration of 50-200 $\mu\text{mol/L}$, The voltage outputs of experimental tests were higher than control tests. That is because low concentration of nickel ion (50-200 $\mu\text{mol/L}$) can play an auxo-action for anaerobic microorganisms. Cai and Wang reported that the low concentration Ni^{2+} promote the growth of anaerobic microorganism in the anaerobic reactors increasing the anaerobic methane production [31, 32]. To our best knowledge, this is the first report of the low concentration Ni^{2+} promoted the electricity generation when feeding the wastewater containing Ni^{2+} into anode of MFCs. While in nickel ion concentration of 200-600 $\mu\text{mol/L}$, the voltage outputs of experimental tests were basic same with control tests. The conclusion illustrated the voltage outputs did not decrease in those concentrations.

Gradient increase of nickel ions (600-1000, stepped by 100 $\mu\text{mol/L}$) were adding into anode of MFCs in order to investigate the maximum tolerance concentration (Fig. 4). Results showed that higher 600 $\mu\text{mol/L}$ (35.21 mg/L) the voltage output of the blank was higher than that of MFCs with Ni^{2+} .

3.2.3 Cadmium

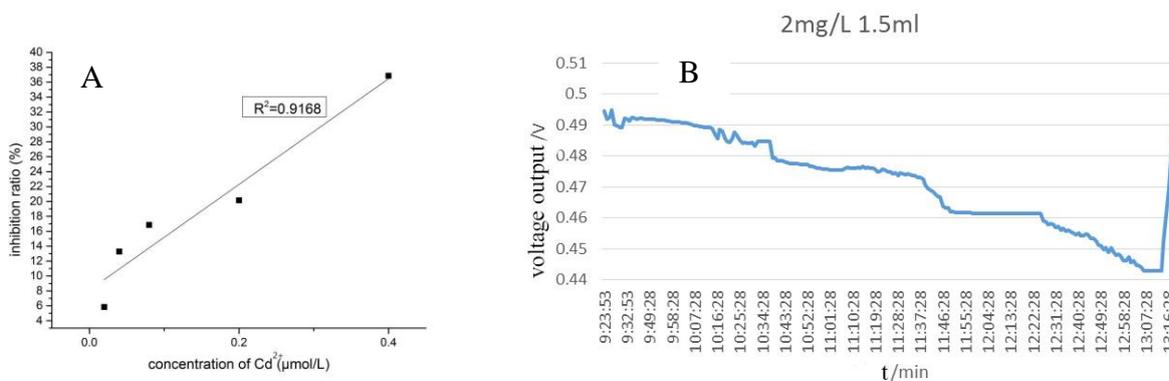


Figure 5. Variation of voltage output in MFCs: A) Inhibition ratio of MFCs with different concentrations of Cd^{2+} ; B) Variation of voltage output in MFC-3 (Cd^{2+})

The research of Cd^{2+} and Hg^{2+} are qualitative. Since the voltage outputs decreased significantly even the concentration of metal ions is very low. The voltage outputs is shown in Fig. 5. Gradient increase of $[\text{Cd}^{2+}]$ from 0.02 $\mu\text{mol/L}$ to 0.4 $\mu\text{mol/L}$, the inhibition ratio of the voltage outputs is from 5.84 % to 36.86 %. And as shown in Fig. 5, the impact of concentration of Cd^{2+} is quiet linear, as the $R^2=0.9168$.

3.2.4 Mercury

The impact of concentration of Hg^{2+} is similar with Cd^{2+} , as the inhibition ratios of 4 MFCs are shown in Fig. 6. Gradient increase of $[\text{Hg}^{2+}]$ from 0.007 $\mu\text{mol/L}$ to 0.14 $\mu\text{mol/L}$, the inhibition ratios of

the voltage outputs are from 1.3 % to 13.4 %, MFC-2 are from 1.72 % to 6.87 %, MFC-3 are from 1.26 % to 10.85 % and MFC-4 are from 1.19 % to 8.03 % (Fig. 7). The R^2 is 0.9588, 0.9721, 0.9632 and 0.9390, the linearity of the data are well. As the data is shown, the impact of Hg^{2+} is lower than Cd^{2+} . Different from the Cu^{2+} and Ni^{2+} , the voltage outputs decreased once the Cd^{2+} and Hg^{2+} add in. Many researchers reported that Cd^{2+} and Hg^{2+} emitted to the natural environment can pose a threat to human and animal health because of their biotoxicity [33]. The voltage output in the MFCs was used as the indication index of the heavy metals' toxicities by Mia [34]. In this research, the voltage outputs decreased significantly with the very low concentrations of Cd^{2+} and Hg^{2+} (even 0.02 $\mu\text{mol/L}$).

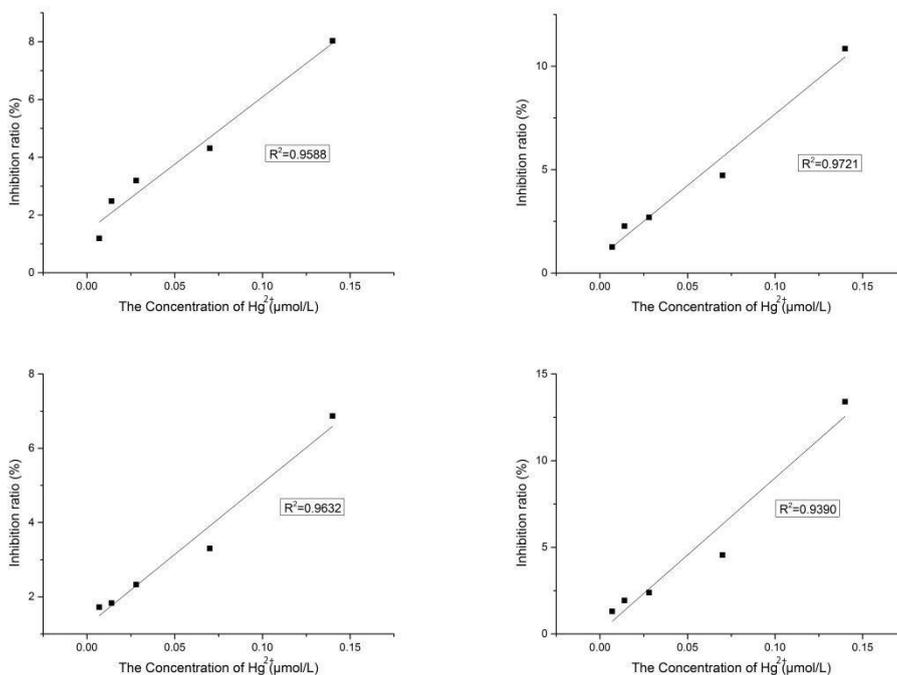


Figure 6. Inhibition ratio of MFCs with different concentrations of Hg^{2+} in 4 batches

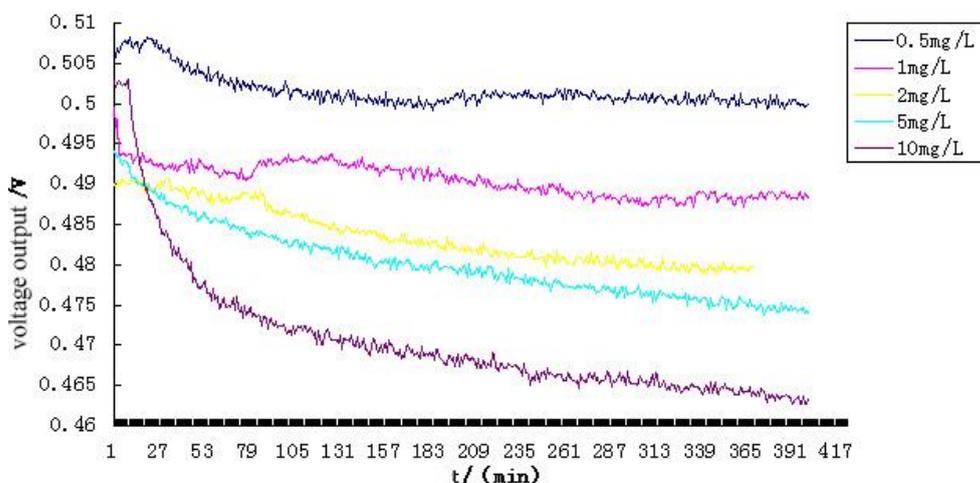


Figure 7. Voltage output of MFC-4 (Hg^{2+}) with different concentrations of Hg^{2+}

3.3 Recovery of voltage output when feeding the anolyte containing no heavy metal ions

3.3.1 Copper

As mentioned above, the voltage output of MFCs was inhibited by Cu^{2+} at high concentrations due to its biological toxicity. After the exposure to high levels of Cu^{2+} , MFC-3 anodic solution was replaced with a refresh solution containing no copper. Upon this change, the maximum voltage output for MFC-3 with a 500 Ω external resistance increased to 0.565 V. This was much higher than MFC-0 (no copper control) of 0.503 V and MFC-4 (anodic solution unchanged, remained at 88.89 mg/mL) of 0.452 V (Fig. 2D). These data confirmed that the voltage output could recover after terminating Cu^{2+} at high concentrations, and that their voltage outputs would increase due to the deposition of Cu^0 on the anodic biofilm.

3.3.2 Nickel

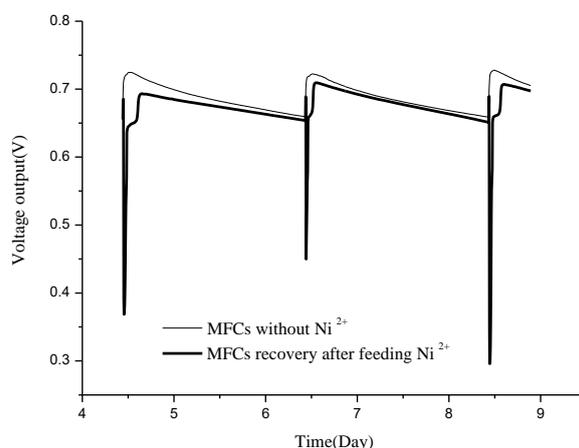


Figure 8. Voltage output after refreshing anolyte and catholyte containing no Ni^{2+} after the MFCs were fed with 1000 $\mu\text{mol/L}$ Ni^{2+} .

The anolyte in the MFCs was replaced with fresh anolyte containing no Ni^{2+} after the MFCs were fed with 1000 $\mu\text{mol/L}$ Ni^{2+} . It can be seen that the difference was due to the beginning period after refreshing anolyte, the nickel ions concentration was high, and inhibit the voltage outputs (Fig. 8). In the two batches, the average voltage outputs of control tests were 0.684 V, while the experimental tests were 0.676 V, the inhibit rate was just 1.17 %. These data confirmed that the voltage output could recover after terminating Ni^{2+} at high concentrations.

3.3.3 Cadmium

Since the MFCs can not recover in a toxic anolyte, the anolyte in the MFCs was replaced with fresh anolyte containing no Cd^{2+} after the experiment in 24 h. In the five batches, the average voltage output after the recover was 0.4940 V, while the average voltage output before the experiment was

0.4960 V, the inhibition rate was 0.4 %. These data confirmed that the voltage outputs in MFCs were recovered in 24 h.

3.3.4 Mercury

The experiment of Hg^{2+} was same to Cd^{2+} . As the mentioned above, the impact of Hg^{2+} is lower than Cd^{2+} , exactly the recover of MFCs with Hg^{2+} was easier than Cd^{2+} . After the anolyte in the MFCs was replaced with fresh anolyte containing no Hg^{2+} , the voltage outputs were recovered in 30 min.

3.4 Impacts of different heavy metal ions

The behaviors of heavy metal ions in MFCs were different. Cu^{2+} and Ni^{2+} were confirmed that they can be acted as a catalyst in MFCs in sometimes, and the data of this paper tallies with this conclusion. Cu^{2+} and Ni^{2+} can improve the performance of MFCs in low concentrations, and as the concentration become higher, the voltage outputs were lower than the control. The inhibition of voltage outputs with Cu^{2+} and Ni^{2+} can be easily explained that the higher concentration of metal ions are toxic to microbial in MFCs. The performance of MFCs with Cd^{2+} and Hg^{2+} are different from those with Cu^{2+} and Ni^{2+} , the voltage outputs were decreased once these metal ions add into the MFCs. This phenomenon shows that Cd^{2+} and Hg^{2+} are toxic to the microbials even in a low concentration.

3.5 Behavior of heavy metal ions in wastewater treatment

The heavy metal can be remove with hydroxide precipitation or as a electron acceptor. After this research about heavy metal ions, we can conclude that MFCs can not work or the performance of MFCs would be worse with a high concentration of Cu^{2+} or Ni^{2+} , the limit concentration of Cu^{2+} is 347 $\mu\text{mol/L}$, and Ni^{2+} is 600 $\mu\text{mol/L}$, while when the concentration of these ions are low ($[\text{Cu}^{2+}]$ is lower than 303 $\mu\text{mol/L}$, $[\text{Ni}^{2+}]$ is lower than 200 $\mu\text{mol/L}$), the performance of MFCs would be better than without these ions. The performance of MFCs would also be worse with Cd^{2+} and Hg^{2+} . So wastewater with a high concentration of these heavy metal ions need a pre-treatment to reduce those ions before MFCs.

4. CONCLUSIONS

This paper studied the effect of heavy metal ions (copper, nickel, cadmium and mercury) on anode, mainly in voltage output and its recovery feeding non-containing heavy metal ion. When feeding the anolyte containing Cu^{2+} of 303 $\mu\text{mol/L}$ or lower, the voltage output of MFCs can be improved, and Cu^{2+} of higher 347 $\mu\text{mol/L}$ decrease the voltage output of MFCs. When feeding the anolyte containing Ni^{2+} of 50-200 $\mu\text{mol/L}$, the voltage output of MFCs can be improved; and Ni^{2+} of

higher 600 $\mu\text{mol/L}$ decrease the voltage output of MFCs. On the other hand, when feeding the anolyte containing Cd^{2+} and Hg^{2+} , the voltage outputs decreased once Cd^{2+} and Hg^{2+} added into the MFCs. As for the recovery of MFCs, after replaced the anodic solution with a refresh solution that containing no heavy metal ions, the MFCs would recover in several hours. Furthermore, as the MFCs can be recovered in a short time, and the voltage outputs would decrease significantly and quickly after the metal ions added into the MFCs, so biosensors constructed by MFCs may be a feasible direction in the later research.

Reference

1. V. M. Ortizmartínez, M. J. Salargarcía, F. J. Hernándezfernández and A. P. D. L. Ríos, *Energy*, 93 (2015) 1748.
2. A. Ter Heijne, F. Liu, R. V. D. Weijden, J. Weijma, C. J. Buisman and H. V. Hamelers, *Environ., Sci. Technol.*, 44 (2010) 4376.
3. H. C. Tao, W. Li, M. Liang, N. Xu, R. J. Ni and W. M. Wu, *Bioresour. Technol.*, 102 (2011) 4774.
4. H. Luo, B. Qin, G. Liu, R. Zhang, Y. Tang and Y. Hou, *Front. Env. Sci. Eng.*, 9 (2015) 522.
5. Y. Li, Y. Wu, B. Liu, H. Luan, T. Vadas and W. Guo, *Bioresour. Technol.*, 192 (2015) 238.
6. Z. Chen, B. K. Zhu, W. F. Jia, J. H. Liang and G. X. Sun, *Environ. Technol. Innov.*, 3 (2015) 63.
7. X. Wang, J. Li, Z. Wang, H. Tursun, R. Liu, Y. Gao and Y. Li, *Environ. Sci. Pollut. Res.*, 23 (2016) 1.
8. N. Touach, V. M. Ortiz-Martínez, M. J. Salar-García, A. Benzaouak, F. Hernández-Fernández and A. P. D. L. Ríos, *Sep. Purif. Technol.*, 171 (2016) 174.
9. L. J. Zhang, H. C. Tao, X. Y. Wei, T. Lei, J. B. Li and A. J. Wang, *Chemosphere*, 89 (2012) 1177.
10. Z. Wang, B. Lim and C. Choi, *Bioresour. Technol.*, 102 (2011) 6304.
11. Z. Lu, D. Chang, J. Ma, G. Huang, L. Cai and L. Zhang, *J. Power Sources*, 275 (2014) 243.
12. S. Kato, K. Hashimoto and K. Watanabe, *Microbes Environ.*, 28 (2013) 141.
13. P. Van der Maas, S. Peng, B. Klapwijk and P. Lens, *Environ. Sci. Technol.*, 39 (2005) 2616.
14. O. Lefebvre, Z. Tan, S. Kharkwal and H. Y. Ng, *Bioresour. Technol.*, 112 (2012) 336.
15. B. Rousseau, X. Dominguez-Benetton, M. L. Délia and A. Bergel, *Electrochem. Commun.*, 33 (2013) 1.
16. K. Zuo, L. Yuan, J. Wei, P. Liang and X. Huang, *Bioresour. Technol.*, 146 (2013) 637.
17. M. A. Rodrigo, P. Cañizares and J. Lobato, *Bioresour. Technol.*, 101 (2010) 7014.
18. D. Chang, H. Zhang, Z. Lu G. Huang, L. Cai and L. Zhang, *Prog. Chem.*, 26 (2014) 1244.
19. G. M. Gadd and A. J. Griffiths, *Microb. Ecol.*, 4 (1977) 303.
20. M. Kim, M. S. Hyun, G. M. Gadd and H. J. Kim, *J. Environ. Monitor.*, 9 (2007) 1323.
21. S. Patil, F. Harnisch and U. Schröder, *ChemPhysChem*, 11 (2010) 2834.
22. N. E. Stein, H. V. Hamelers and C. N. Buisman, *Sensor. Actuat. B: Chem.*, 163 (2012) 1.
23. K. Rabaey, W. Ossieur, M. Verhaege and W. Verstraete, *Water Sci. Technol.*, 52 (2005) 515.
24. S. Choi, J. R. Kim, J. Cha, Y. Kim, G. C. Premier and C. Kim, *Bioresour. Technol.*, 128 (2013) 14.
25. S. Kondaveeti, J. Lee, R. Kakarla, H. S. Kim and B. Min, *Electrochim. Acta*, 132 (2014) 434.
26. H. Liu and B. E. Logan, *Environ. Sci. Technol.*, 38 (2004) 4040.
27. S. S. Şengör, P. Gikas, J. G. Moberly, B. M. Peyton and T. R. Ginn, *J. Chem. Technol. Biot.*, 83 (2012) 374.
28. X. H. Zhou, T. Yu, H. C. Shi and H. M. Shi, *Water Res.*, 45 (2011) 953.
29. V. Ochoa-Herrera, G. León, Q. Banihani, J. A. Field and R. Sierra-Alvarez, *Sci. Total Environ.*, 412 (2012) 380.
30. M. Y. Pamukoglu and F. Kargi, *Microb. Tech.*, 40 (2007) 1228.

31. X. Cai, Z. J. Hong, R. H. Dai, Y. Liu and X. Liu, *Water Sci. Technol.*, 65 (2012) 456.
32. L. J. Wang, H. Y. Zhang, Y. L. Tian and F. J. Zhang, *Adv. Mat. Res.*, (2013) 805, 219.
33. A. S. Mathuriya and J. V. Yakhmi, *Environ. Chem. Lett.*, 12 (2014), 483.
34. K. Mia, H. Moonsik, G. M. Gadd and K. Hyungjoo, *J. Environ. Monit.*, 9 (2007), 1323.

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