Microbial fuel cell coupled constructed wetland promote the further degradation of azo dye decolorization products

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Abstract

Microbial fuel cell coupled constructed wetland (CW-MFC) was constructed for azo dye decolorization and further degradation. Methyl Orange (MO) was chosen as the model azo dye due to the simple decolorization products. The decolorization products were analyzed qualitatively via GC-MS and the N,N-Dimethyl-p-phenylenediamine (DMPD) was chosen as one of the MO decolorization products to study the further degradation performance of decolorization products in the CW-MFC. 1,4-Benzenediamine, N-methyl- and p-Phenylenediamine (PPD) was another intermediate product of MO decolorization. Electrical characteristics of CW-MFC were studied to evaluate the interplay between the azo dye degradation and the electricity generation. The MO decolorization rate of open and closed-circuit CW-MFC were 75.59% and 87.60%, respectively, while the DMPD degradation rate in the anode layer of open and closed-circuit CW-MFC were 84.96% and 96.33%, respectively. In the anode layer, suitable anode materials can promote the further degradation of azo dye degradation products, while in the

cathode layer, close current promoted the further degradation of the decolorization products.

Key words

microbial fuel cell, constructed wetland, Azo dye, decolorization products, further degradation

1. Introduction

In recent years, more and more Microbial fuel cells (MFCs) have been used in wastewater treatment because a wide variety of organic matter present in wastewater can be used as renewable resource to electricity production (Virdis and others 2010). Furthermore, it has been demonstrated that the microorganisms of MFCs can degrade many types of toxic and refractory compounds (Luo and others 2011; Zhang and others 2009). In recent years MFCs have been embedding into sediments and wetlands to treat wastewater and becoming a kind of new technology (Zhao and others 2013). Shrivastava et al. (Shrivastava and others 2015) had reported a kind of wetland MFC for wastewater treatment.

Azo dyes wastewater may lead to a series of environmental problems (de Aragão Umbuzeiro and others 2005). Studies have shown that azo dyes are biorefractory organics and have ecotoxicity (Chung and Stevens 1993). Azo dyes can be decolorized in anaerobic environment and yield aromatic amines which were also biorefractory compounds and harmful to aquatic plants and animals (Méndez-Paz and others 2005). Some aromatic amines were carcinogens and teratogen for human.

Since few studies have attempted to characterize the performance of MFCs in azo dye decolorization products treatment, it was generally accepted that on the anode of MFCs, azo dyes were reduced to aromatic amines which were supposed to non-biodegradable under the anaerobic environment of anode (Van der Zee and others 2001). Therefore the decolorization in anode have gained considerable attention, however, the further degradation of decolorization products in MFCs were involved little. Although some studies (Fernando and others 2014; Li and others 2010b) have mentioned the further degradation of azo dye decolorization products, some limitations and shortcoming still exist. Firstly, they were all assumed that the aromatic amines can only be oxidized in the aerobic part of MFCs such as the cathode. Secondly, the analyses of decolorization products were mainly qualitative analysis and the further degradation of these products were less studied. These were disadvantages to make an in - depth study about the effect of MFCs in wastewater treatment.

Kahng et al. (Kahng and others 2000) reported a novel microorganism called HY99, which could degrade aniline under both aerobic and anaerobic environments. And it has been reported that the p-nitrophenol was completely removed in the anode chamber of MFCs while electricity was concomitantly generated (Liu and others 2013). This illustrated that the degradation of aromatic amines on the anode and the reduction reaction of biorefractory organics on the cathode were possible.

In this study, microbial fuel cell coupled constructed wetlands (CW-MFC) have been established and been used to azo dye decolorization and the further degradation

of decolorization products. Glucose was used as the co-substrate because the bacteria in the CW-MFC can't degrade the azo dye without co-substrate. Methyl Orange (MO) has been chosen as the model azo dye because of its relatively simple decolorization products and high oxidation-reduction potential (Liu and others 2009) which made it easy to study the degradation process of MO. The degradation process of MO in the anode and cathode layer of the CW-MFCs was studied and the decolorization products of MO were analyzed by GC-MS. Then we chose N, N-Dimethyl-p-phenylenediamine (DMPD) as one of the MO decolorization products, analyzed the degradation of DMPD in the anode and cathode layer of the CW-MFC via quantitative analysis. According to above results, the influence of close current on the further degradation of decolorization product was evaluated.

2. Material and methods

2.1 Synthetic wastewater

Commercial pure Methyl Orange ($C_{14}H_{14}N_3SO_3Na$, 327.33) was purchased from Shenyang Xin Guang Chemical factory, China. The glucose (50mg/L) was used as carbon source and was added into the medium solution to make glucose synthetic wastewater, while the MO (450mg/L) and glucose (50mg/L) was added into the medium solution to make MO synthetic wastewater. The medium solution in this study was made of tap water, and the composition was as following: NH_4Cl (0.31 g/L), NaH_2PO_4 (4.97 g/L), Na_2HPO_4 (2.75 g/L), KCl (0.13 g/L), $NaHCO_3$ (3.13 g/L) and 0.1 mL concentrated trace element solution as reported by Liu (Liu and others 2008).

2.2 CW-MFC system construction

By embedded the anode and cathode into a constructed wetland (CW), a CW-MFC system was constructed (Fig. 1). The CW-MFC was constructed in a polyacrylic plastic cylinder with an internal diameter of 30 cm and a length of 52.5 cm. There were four layers: the bottom layer (20-cm high) was made of gravel, the MFC anode layer (10-cm high) was made of granular activated carbon (GAC, GAC is a kind of frequently used MFC electrode material because of its good electrical conductivity). The middle gravel layer (20-cm high) was made of gravel, and the MFC cathode layer (2.5-cm high) was made of GAC. Stainless steel mesh was buried into the anode and the cathode to improve the electrical conductivity. The cathode was left open to air. The anode was leaded out by titanium wire passing through the reactor and connected with cathode by copper conductors with a resistor of 1000 Ω . Because the internal resistances of SMFCs and CW-MFCs were very large (nearly 1000 Ω , or more large), the external resistance of 1000 Ω can maximizing CW-MFC performance. Ten sampling ports were arranged at an interval of 5cm throughout the height of the reactor to collect samples from different depths.

Figure 1. The structure of the CW-MFC: (A) The model of the CW-MFC; (B) The

photograph of CW-MFC.

2.3 Inoculation, system start-up and experiments

In order to reduce the effects of some other factors such as biological parameters, there were 9 reactors divided into 3 parallel groups, each group had 3 reactors. The experimental results were the average values of these 3 parallel groups. In every group, one open-circuit CW-MFC and two closed-circuit CW-MFCs were operated to establish a reference frame. Concentrated anaerobic sludge collected from the East City Municipal Wastewater Treatment Plant (Nanjing, China) was used as the inoculum source and poured into the anode of three reactors. The glucose synthetic wastewater was pumped continuously into the reactors from the bottom after inoculation. The hydraulic retention time (HRT) was 3 days. A data acquisition module (DAM-3057 and DAM-3210, Art Technology Co. Ltd., China) was used to collect voltage data of closed-circuit reactors. And a calomel reference electrode (SCE) was used as reference electrode to test the anode and cathode electrode potential. Systems were operated for several days after the maximal reproducible voltages were obtained. Then MO synthetic wastewater was continuously pumped in to the open-circuit reactor and one of the closed-circuit reactors, while the glucose synthetic wastewater was still pumped in to another closed-circuit reactor. The systems were then keeping running for over 2 months after the maximal reproducible voltages were observed again. Two experiments were conducted to investigate the degradation mechanism of MO in the CW-MFC: (1) For the open and closed-circuit CW-MFCs with MO synthetic wastewater, the MO concentrations and

the COD concentrations of different parts of CW-MFCs were measured. The decolorization products were detected and the DMPD concentrations were measured by Gas Chromatograph-Mass Spectrometer (GC-MS). (2) For two closed-circuit CW-MFCs with glucose and MO synthetic wastewater, respectively, the electrical characteristics were studied. All of the experiments were repeated 3 times and error bars were used to represent standard errors of each parallel experiment.

2.4 Analytics and calculations

The MO concentration was measured by spectrophotometry (Labtech 9100B PC, US). Samples were filtered through a 0.45- μ m syringe filter to remove suspended solids. MO concentration was determined by standard curve at a maximum wavelength of 538 nm. The decolorization rate of MO was calculated according to the following formula:

$$\text{Decolorization rate \%} = (A-B)/A \times 100\% \quad (1)$$

A is the initial absorbance while B is the observed absorbance.

The COD was measured via potassium dichromate method (APHA method 5220) and the COD removal rate was calculated according to the following formula:

$$\delta\text{COD} = (\text{COD}_{\text{in}} - \text{COD}_{\text{out}}) / \text{COD}_{\text{in}} \times 100\% \quad (2)$$

COD_{in} and COD_{out} represent the initial COD concentration (mg/L) and the observed COD concentration (mg/L) at different depths, respectively.

Oxidation-Reduction Potentials (ORP) of different parts of the CW-MFCs were measured by ORP determinator (IM32P, DKK-TOA Co. Ltd., Japan).

GC-MS (ITQ 1100, Thermo Fisher Scientific Co. Ltd., USA) was used to

detect the MO decolorization products. 100mL water samples were extracted with CH_2Cl_2 , and concentrated to 1mL, and test with DB-5MASS capillary chromatogram column, temperature programming, and ion trap mass spectrometer. The carrier gas was high purity helium and the flow rate was 1mL/min. The temperature program was as following: the gasification compartment temperature was firstly set to 60°C for 0.5min, and then raised linearly to 235°C at a rate of 25 °C /min. After maintaining at 235°C for 2min, it was further raised linearly to 250°C at a rate of 2°C /min and then maintained at 250°C for 5min. Finally, the temperature of gasification component was increased linearly to 280°C at a rate of 15°C /min and maintained for 5min. The molecular weight was scanned in the range of 45 m/z ~ 600 m/z. The retention time and sampling volume was 4 min and 1 μL , respectively. The sample was injected with injector. The ionization way of mass spectroscopy was electron impact (EI) with the voltage of electron multiplier at 1605V. The temperatures of the ion source and the interface were 230°C and 280°C, respectively. Separated ingredients were analyzed with a reference to the NIST MS Search 2.0mass spectral library database. The analysis of DMPD used GC-MS choice ion pattern (SIM). The temperature program was the same as above. The peak areas of 93 m/z, 121 m/z, and 136 m/z were used to generate standard curve.

Because cyclic voltammetry was not suitable for sediment MFC (SMFC) and CW-MFC, the electrochemical characteristics of the CW-MFC were determined as follows.

The volumetric power density (P_v , W/m^3) was calculated by the effective

volume of the anode chamber (m^3) because the electrons used to power generation was generated in anode (Li and others 2010b). The power density curve and the polarization curve were employed to evaluate the electrical characteristics of CW-MFCs. The internal resistance (R_i) was calculated by the linear region of the polarization curve. The anode coulombic efficiency (CE) was calculated as follows:

$$CE = \frac{I}{F \times \frac{4}{32} \times Q_{\text{in}} \times \Delta\text{COD}} \quad (3)$$

I is the current (A), F is the Faraday constant ($96485 \text{ C/mol} \cdot \text{e}^-$), 32 is the molar mass of O_2 , 4 is the electron number gained by the reduction reaction of 1 molar O_2 , Q_{in} is volumetric influent flow rate of CW-MFCs (L/s), ΔCOD is the difference of chemical oxygen demand (COD) between the influent and the effluent of the anode.

T-text was employed to compare data between different groups. P values < 0.05 indicated that there were differences between the groups. P values > 0.05 indicated no significant differences between groups.

3. Results and discussion

3.1 The MO decolorization and the COD removal

Following a successful acclimation period of 2 months after the MO wastewater was pumped into CW-MFCs, the MO decolorization and the COD removal rates of CW-MFCs were stable. As shown in Fig. 2A, the decolorization happened mostly in the anode (sampling port 5~7). The MO concentration decreased from 284.82 mg/L to 38.01mg/L in the anode of the closed-circuit CW-MFC and the decolorization rate was 69.41%. The MO concentration decreased from 284.87 mg/L to 95.35mg/L in the anode of the open-circuit CW-MFC and the decolorization rate

was only 53.30%. In the bottom layer (sampling port 1~4) of the open-circuit CW-MFC, the MO concentration decreased from 355.58 mg/L to 284.87 mg/L while in the closed-circuit CW-MFC the change was from 355.58 mg/L to 284.82 mg/L. The decolorization rates were 18.10% and 18.46%, respectively. According to T-text, there were no significant differences between open and closed-circuit CW-MFC (P value > 0.05). In the middle layer and the cathode layer (sampling port 8~11) of the CW-MFCs, the MO concentrations of two CW-MFCs didn't change significantly according to the T-text (P value > 0.05). Indicating the middle layer and the cathode layer had no contribution to the decolorization. The COD concentrations of two CW-MFCs were shown in Fig. 2B. The changing trends of COD concentrations in the bottom layer and the anode layer of two CW-MFCs were similar to the MO concentrations. In the bottom layer of open and closed-circuit MFC the COD concentrations decreased from 455.47 mg/L to 386.17 mg/L and 391.29 mg/L, respectively. And the COD removal rates were 15.62% and 14.59%, respectively. There were no significant differences between open and closed-circuit CW-MFC (P value > 0.05). In the anode layer of the closed-circuit MFC, the COD concentration decreased from 391.29 mg/L to 274.30 mg/L, and the COD removal rate was 25.69% while the COD removal rate of the open-circuit CW-MFC was only 15.97%. In the middle layer and the cathode layer, the COD concentration of open-circuit CW-MFC didn't change significantly according to the T-text (P value > 0.05), and was remained around about 300.00 mg/L. On the contrary, the COD concentration of closed-circuit CW-MFC was decreased from 274.30 mg/L to 199.47 mg/L.

Figure 2. The MO and the COD concentration of CW-MFCs: (A) The MO concentration of the open and closed-circuit CW-MFC; (B) The COD concentration of the open and closed-circuit CW-MFC.

It is clear from these data that the anode layer made the most contribution to both decolorization and COD removal. The decolorization rate and the COD removal rate in the anode layer of the closed-circuit CW-MFC were higher than the open-circuit CW-MFC. Research suggested that the electric field of closed-circuit CW-MFC can promote organics degradation by changing the physiological characteristics of microorganism (Bencheikh-Latmani and others 2005; Ringeisen and others 2006; Srivastava and others 2015), and promote the reduction reaction by

increase the number of electrons (Sun and others 2009). To realize the azo dye decolorization in the MFCs, there must be two essential conditions. The first is sufficient co-substrate and microbial biomass (Khehra and others 2006), the second is appropriate oxidation-reduction potential. Study showed azo dyes can only be reduced when the oxidation-reduction potential was less than -50 mV (Bromley-Challenor and others 2000).

In this study, the wastewater was pumped into the reactors from bottom, that means the concentration of co-substrates which was indispensable to the azo dye decolorization and microbial growth in the bottom layer was higher than the middle layer. This had been demonstrated in our previous study (Fang and others 2015) and may be the reason for the decolorization rate of bottom layer was higher than the middle layer. Besides, the directly enhancements in decolorization and COD removal rates of the last sampling ports may attribute to the slow influent speed which made the fresh wastewater diluted by the wastewater in the reactors.

In the middle and the cathode layer of the two CW-MFCs, the MO couldn't be decolorized because to broke azo bonds need an environment ORP less than -50 mV, the cathode layer of CW-MFCs were left open in the air, the O₂ in the air will enhance the ORP of the cathode and the middle layer. The ORP of the middle and cathode layer was between 5 mV and 138 mV as showed in Fig. 3. Therefore, in this study, azo dyes couldn't be decolorized in the middle and the cathode layer. Likewise, the COD concentration of the open-circuit CW-MFC remained unchanged in the middle layer and the cathode layer indicated these two layers did no

contribution to the COD remove. Significantly, in the effluent of the closed-circuit CW-MFC, the COD concentration was decreased. This indicated the wastewater was further degraded by the cathode. Studies have shown the decolorization products can be oxidized and degraded under aerobic conditions (Li and others 2010a; Lourenço and others 2006). On the other hand, some refractory compounds can also be reduced by the cathode of MFCs (Kong and others 2013; Shen and others 2012). In this study, these two functions may existence simultaneously. The closed-circuit system promoted the further degradation of azo dye wastewater.

Figure 3. The oxidation-reduction potential (ORP) of CW-MFCs.

3.2 The degradation of MO decolorization products

In order to investigate the promotion of CW-MFC in decolorization products degradation, samples from different parts of CW-MFCs were analyzed by GC-MS. The sample from influent was chosen as a reference. As shown in Fig. 4A, there was no obvious peak in gas chromatography scheme. According to the standard reference and the mass spectrum, the peaks in the scheme were impurities in the column. We collected water samples from sampling port 5 (in front of the anode), sampling port

7 (behind the anode) and the effluent. Fig. 4B and Fig. 4C were the samples collected in front of the anode of closed-circuit and open-circuit CW-MFC, respectively. There was a sharp peak (peak A) eluted at about 12.89 min in each scheme. Fig. 4H was the mass spectrum of peak A, what was N,N-Dimethyl-p-phenylenediamine (DMPD) according to the standard reference and previously reported studies (Cai and others 2012),. The gas chromatography of the samples collected behind the anode of closed-circuit and open-circuit CW-MFC were shown at Fig. 4D and Fig. 4E. There were two more peaks (peak B and peak C) beside peak A, eluted at about 11.58 min and 12.80 min, respectively. Fig. 4I and Fig. 4J were the mass spectrum of these two peak, according to the standard reference, they were 1,4-Benzenediamine, N-methyl- and p-Phenylenediamine (PPD), respectively. Fig. 4F and Fig. 4G were the gas chromatography of the effluent of closed-circuit and open-circuit CW-MFC. In Fig. 4F there were only two peaks: peak A and B, in Fig. 4G, there were still 3 peaks: peak A, B and C.

Figure 4. The gas chromatography scheme and the mass spectrum of the decolorization products: (A) Influent; (B) Water sample in front of the anode of the closed-circuit CW-MFC; (C) Water sample in front of the anode of the open-circuit CW-MFC; (D) Water sample behind the anode of the anode of the closed-circuit CW-MFC; (E) Water sample behind the anode of the anode of the open-circuit CW-MFC; (F) The effluent of closed-circuit CW-MFC; (G) The effluent of open-circuit CW-MFC. (H) The mass spectrum of DMPD; (I) The mass spectrum of PPD; (J) The mass spectrum of 1, 4-Benzenediamine, N-methyl-.

It is necessary to quantify the decolorization products to meet the demand of mechanism study. Therefore, DMPD was selected as one of the major decolorization products and be quantified to provide data support for the degradation mechanism study. Fig. 5 was the DMPD concentration in different parts of open and closed-circuit CW-MFC. In the influent (sampling port 0), there were no DMPD in the water sample. In the bottom layer of the two CW-MFCs (sampling port 1~5), the DMPD concentrations were about 21.00 mg/L and 19.00 mg/L, respectively. According to the T-text, there were no significant differences between open and closed-circuit CW-MFCs in the bottom layer (P value > 0.05). When the water passed through the anode layer, the DMPD concentrations of the open and closed-circuit CW-MFC decreased to 18.20 mg/L and 5.44 mg/L, respectively. The decrease of closed-circuit CW-MFC was larger than the open-circuit CW-MFC. When the water passed through the middle layer, the DMPD concentrations of the open-circuit and the closed-circuit CW-MFC were 19.16 and 5.15 mg/L, respectively.

And didn't change significantly compare to the DMPD concentrations when the water passed through the anode layers according to the T-text (P values > 0.05). In the effluent, the DMPD concentrations of the open-circuit CW-MFC was 18.42 mg/L. According to T-text, the DMPD concentration did not significantly change in the cathode layer (P value > 0.05). However, in the effluent of the closed-circuit CW-MFC the DMPD concentration was decreased to 2.49 mg/L.

Figure 5. The quantitative analysis of N,N-Dimethyl-p-phenylenediamine (DMPD).

It has been confirmed that the azo bond of azo dye could be broken and yield aromatic amines (Watts and others 2006). As mentioned before, one molecule of MO would be reduced to one molecule of DMPD and one molecule of p-Anilinesulfonic Acid (4-ABS). Because the 4-ABS is not volatile organic, it can't be detected by GC-MS (Murali and others 2013). Therefore in the gas chromatography scheme of this study there were only DMPD peaks. For both close and open-circuit CW-MFC, according to Fig. 4A, B, C and Fig. 2A, there was no DMPD in the influent, and DMPD has been detected in the bottom layer while the decolorization began to happen in that layer too. According to previous research, this

indicated the decolorization of MO generated DMPD (Cai and others 2012; Li and others 2013).

After the water passed through the anode layer of two CW-MFCs, there were two more chemical substances detected both in the close and the open-circuit CW-MFC: PPD and 1, 4-Benzenediamine, N-methyl-. DMPD has two methyl groups connect to the para-position amino group, while the number of the methyl groups of 1, 4-Benzenediamine, N-methyl- was 1 and PPD has no methyl group. The result indicated that DMPD was further degraded in the anode layer. According to the molecular formulas, the mechanism degradation of DMPD was hydrogenation and demethylation, and there were two probable degradation pathways. DMPD may firstly remove a methyl group to yield 1, 4-Benzenediamine, N-methyl-, and then remove the other methyl group to yield PPD. Or DMPD may remove one or two methyl groups to yield 1, 4-Benzenediamine, N-methyl-, and PPD at the same time, and the 1, 4-Benzenediamine, N-methyl- may be further degraded to yield PPD. Compared to other researches that study the anaerobic decolorization of MO, the decolorization products were only DMPD and 4- ABS (Cai and others 2012; Chen and others 2008; Murali and others 2013), the other two compounds haven't been reported before. This may because of the anode layer of this study was filled with GAC while the anode of the researches mentioned before were made of carbon paper, carbon fibre or carbon felt, and the anode chamber of these researches were filled with liquid. Studies have confirmed that the surface oxygen-containing groups of activated carbon can affect the catalytic performance and change the

oxidation-reduction characteristic and promote the reduction reaction (Almarri and others 2009). In this study, no 1, 4-Benzenediamine, N-methyl- or PPD were detected in the bottom layer despite there was DMPD existence. The bottom layer was not filled with GAC, which can also testify the function of GAC in DMPD degradation. This indicated select suitable anode materials and configuration may promote the further degradation of azo dye decolorization products, this will benefit the further study of complete degradation of azo dyes and some aromatic amines in the anode of MFCs.

The operational conditions of the open and closed-circuit CW-MFC were all the same except the closed-circuit CW-MFC was a closed-circuit system. The differences of experimental results were all appeared in the anode layer and the cathode layer.

In the bottom layer of the two CW-MFCs, according to the decolorization of MO, about 67.50 mg/L MO has been decolorized to yield about 28.05 mg/L DMPD. According to the DMPD quantitative experiment, the measured DMPD concentrations of the two CW-MFCs were 21.92 mg/L and 19.38 mg/L, respectively. Considering the MO was commercial pure and the experimental errors, these results were reasonable. P values (> 0.05) indicated there were no significant differences in the bottom layer between the two CW-MFCs.

In the water sample collected behind the anode layer, the DMPD concentrations of two reactors were very low. However, the decolorization rates of anode layers were pretty high, which indicated there were more DMPD generated in the anode

layers. For the open-circuit CW-MFC, there should generated 99.65 mg/L DMPD, while the closed-circuit CW-MFC should generated 129.78 mg/L DMPD. Plus the DMPD came from the bottom layer there should be 121 mg/L and 148.07 mg/L DMPD in the anode layer of open and closed-circuit CW-MFC, respectively. Therefore the DMPD removal rates in the anode of open and closed-circuit CW-MFC were 84.96% and 96.33%, respectively. The closed-circuit promoted the degradation of DMPD in the anode layer. This may attribute to the acceleration of electron flow caused by current circuit.

In the middle and the cathode layer of the two CW-MFCs, the ORP conditions were all suitable for DMPD reduction. The oxidation-reduction potential of the middle layer was about 5~ 30 mV while the oxidation-reduction potential of open and closed-circuit CW-MFC were 138 mV and 112 mV, respectively (Fig. 3), and the reduction potential of DMPD was about 120 mV vs. SCE (Maleki and Nematollahi 2013). According to the decolorization rates and the measured DMPD values, no MO was decolorized and no DMPD generated in the middle layers of the two CW-MFCs. However, in the cathode layer, the DMPD concentration of closed-circuit CW-MFC was still decreased while the DMPD concentration of open circuit CW-MFC was remain unchanged. This indicated in the cathode of closed-circuit CW-MFC, DMPD can be further degraded. Besides, the 1, 4-Benzenediamine, N-methyl- could also be degraded in the cathode layer of the closed-circuit CW-MFC. According to previous studies, biorefractory organics can be reduced (Liu and others 2013; Wang and others 2013) in the cathode of MFCs by

the electrons passed by the circuit from the anode. In this study, the only difference between the open and closed-circuit CW-MFC was the electric circuit. Therefore, the differences of the experimental results in the cathode layer of the open and closed-circuit CW-MFC was mostly contributed by electric current and electric field caused by the closed-circuit system of the closed-circuit CW-MFC. The most likely explanation was the electrons passing by the circuit from the anode to the cathode promoted the reduction of the DMPD in the cathode. And the ORP was not the only condition of the further degradation of DMPD in the cathode layer.

However, according to the COD and the GC-MS results, there were still some decolorization products haven't been degraded completely. We will further improve the degradation performance of CW-MFC in our future researches.

3.3 The electrical characteristics of CW-MFCs

A variable resistor with a range of 5 Ω to 100,000 Ω was employed in this work to study the electrical characteristics of CW-MFCs with glucose and MO synthetic wastewater, respectively. The glucose concentration of the glucose CW-MFC and MO CW-MFC was the same (50 mg/L) because the co-substrate concentration must be the same in order to evaluate the electrical characteristics. Fig. 6A showed the power density curve and polarization curve of two CW-MFCs. The highest maximum power densities of the glucose and MO CW-MFC were 0.688 W/m³ and 0.081 W/m³, respectively. While the internal resistances of the glucose and MO CW-MFC were 77.095 Ω and 109.270 Ω , respectively. The anode coulombic efficiency of the glucose and MO CW-MFC were 31.47% and 0.19%, respectively.

These figures showed that the electricity generation of the glucose CW-MFC was much better than the MO CW-MFC.

Figure 6. Electricity research of glucose CW-MFC and MO CW-MFC: (A) The polarization and power density curves; (B) Electrode potential.

Studies have shown that the azo dyes and their decolorization products may hinder the growth of electrogenic bacteria because of toxicity (Yue and others 2008), which may reduce the electricity generation capability of CW-MFC. On the other hand, the decolorization of MO and the degradation of DMPD were all need electrons from glucose. As mentioned before, the anode contributed most to MO decolorization and the degradation of decolorization products. The anode coulombic

efficiency of the glucose CW-MFC was much higher than the MO CW-MFC. Firstly, MO can't be used for electricity generation. Secondly, the decolorization of MO and the degradation of DMPD consumed a part of electrons. Therefore, fewer electrons reached the anode of the MO CW-MFC. These weakened the electricity generation of MO CW-MFC. The internal resistance of MFCs constitute by ohmic resistance and polarization resistance (Manohar and Mansfeld 2009). Ohmic resistance comes from electrolyte and electrodes (Du and others 2007). The electrolyte solution and electrodes of glucose and MO CW-MFC were the same. Therefore, the increase of the MO CW-MFC internal resistance was caused by the polarization resistance. According to Fig. 6B, the slope of the anode and the cathode polarization curve of the MO CW-MFC were steeper than the glucose CW-MFC. Indicated the electrode polarization of MO CW-MFC was more seriously than the glucose CW-MFC. Study has shown the electrode polarization was possibly due to a limitation of electron supply to the electrodes (Sun and others 2009). The decolorization of MO and the degradation of the decolorization products on the electrodes were the reason of the internal resistance enhancement of MO CW-MFC. Though the electrical characteristy of MO CW-MFC was not good, the purpose of azo dye degradation has been reached.

4. Conclusions

In the anode layer of CW-MFC, MO was decolorized via hydrogenation and the decolorization product DMPD was further degraded under the anaerobic environments via demethylation to yield PPD. The electric current of closed-circuit

CW-MFC promoted the decolorization of MO and the degradation of DMPD. The decolorization of MO in the closed-circuit CW-MFC was 87.60%, while that in the open-circuit CW-MFC was 75.59%. The removal rate of DMPD in the anode of closed-circuit CW-MFC was 96.33%, while that in the anode of open-circuit CW-MFC was 84.96%. The GAC in the anode layer promoted the further degradation of DMPD. In the cathode of closed-circuit CW-MFC, the DMPD and the 1, 4-Benzenediamine, N-methyl- were further degraded because of the electron passed by circuit. MO and its decolorization products had negative effects on the electricity generation of CW-MFC. However the electricity generation can promote the MO decolorization and the degradation of the decolorization products, and this is the purpose of this study. Further researches were needed to promote the performance of CW-MFC and investigate the degradation mechanism of refractory compounds.

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