Anode-biofilm electron transfer behavior and wastewater treatment under different operational modes of bioelectrochemical system

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HIGHLIGHTS

- Anode-biofilm ET rate differs greatly in different BES operational modes.
- Bioelectroactive species with different formal potentials function as ET mediators.
- The amount of available bioelectroactive species determines anode-biofilm ET rate.
- MEC is the only feasible BES operational mode for advanced wastewater treatment.

ABSTRACT

Anode-biofilm electron transfer behavior was investigated during the advanced wastewater treatment process by three bioelectrochemical systems (BESs): microbial fuel cell (MFC), MFC operated under short circuit condition (MSC), and microbial electrolysis cell (MEC). Under different operational modes, current produced by the anode biofilm varied from 0.92, 4.15 to 8.21 mA in the sequence of MFC, MSC and MEC, respectively. The cyclic voltammetry test on the anode biofilm suggested that the current generation was achieved via various bioelectroactive species with formal potentials at 0.473, 0.402 and 0.345 V (vs. SCE). Gibbs free energy and charge transfer resistance data demonstrated that different amounts of available bioelectroactive species functioned in different BESs. The comparative investigation among MFC, MSC and MEC suggested that MEC was the only feasible operational mode for advanced wastewater treatment, because of its superior current generation capability.

1. Introduction

Microbial fuel cell (MFC) draws wide interest because of its capacity in terms of electricity generation. Based on the biocatalysis characteristic of MFC, many other similar bioelectrochemical systems (BESs) have also been developed for different applications (Liu et al., 2005; Cao et al., 2009; Choi et al., 2012). Wastewater treatment has been successfully realized and proposed to be a promising application in the BES field (Du et al., 2007; Zhu et al., 2013). During the wastewater treatment process, various factors including BES operational mode play important roles on the treatment efficiency (Lorenzo et al., 2010; Mohan and Srikanth, 2011; Erable et al., 2011). Changes in the BES operational mode significantly affect the bacterial kinetics and current generation, which further influence the wastewater removal efficiency (Erable et al., 2011).

In general, depending on the link status between the anode and cathode, there are three BES operational modes developed for wastewater treatment. MFC, whose anode and cathode are linked by an external resistance, is the most common BES operational mode. In MFC, wastewater treatment can be realized coupled with power generation (Liu et al., 2004; Choi et al., 2012). Another kind of BES operational mode is that the anode and cathode are short-circuited (without external resistance), which is denoted as MSC for short. This kind of BES has been utilized for the enhancement of swine wastewater treatment (Xu et al., 2011). In order to further improve the wastewater removal efficiency, microbial electrolysis cell (MEC) has also been employed (Wang et al., 2013; Wen et al., 2013). Generally, wastewater treatment can be improved in the order of MFC, MSC and MEC, because of their current generation increases also in the same order. Current generation has been demonstrated to be a decisive variable for wastewater treatment. Moreover, the current is directly determined by the bioelectrochemical characteristic of biofilm attached on the anode. Therefore, a clear understanding of the anode biofilm characteristic...
is important for the future application of BES in wastewater treatment.

In BES, current is generated from a continuous electron flow which is originally extracted from the anodic substrate by anode-respiring bacteria (ARB). Thus, the electron transfer (ET) rate largely relies on the electrochemical behavior of ARB (or ARB biofilm). Bioelectroactive species (e.g., outer membrane cytochromes, conductivity pili and extracellular excretions) play a crucial role on the ET between the ARB biofilm and the anode (Richter et al., 2009; Gorby et al., 2006; Newman and Kolter, 2000). Besides the biofilm itself (e.g., community, Torres et al., 2009), some abiotic factors can also influence the performance of bioelectroactive species during ET process (Qiao et al., 2008; Busalmen et al., 2008). For example, oxidative and reductive peaks in the cyclic voltammetry of the anode biofilm varied a lot as a function of pH (Qiao et al., 2008). In addition, Geobacter biofilm with various outer membrane cytochromes produced different currents under different electrode potentials (Fricke et al., 2008). Therefore, the ET behavior of the anode-biofilm is considered to be a helpful tool for understanding the fact that current generation differs greatly among MFC, MSC and MEC.

This study aims to investigate the anode-biofilm ET behavior during the wastewater treatment process under different BES operational modes. In addition, a comparative study of advanced treatment of non-degradable real wastewater by cathodic bioelectro-Fenton process (Feng et al., 2010a,b) was carried out among these modes. Current generation, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were recorded for investigating the anode-biofilm ET behavior. Total organic carbon (TOC) was determined to evaluate the feasibility of advanced wastewater treatment by different BESs.

2. Methods

2.1. Reactor configuration

Dual-chamber bioelectrochemical reactor consisting of two identical cylindrical chambers (100 mL for each) was employed in this study. The two chambers were separated by a cation exchange membrane (Zhejiang Qianqiu Group Co., Ltd., China). Graphite felt with a projected surface area of 23.7 cm² was used as the anode and cathode (about 3 cm distance between them).

2.2. Advanced wastewater treatment and electrochemical measurement of the anode biofilm

To obtain basically identical anode biofilms, five reactors were operated under the MFC mode with mixed sludge acquired from a coking wastewater treatment plant (Shaoquan Iron & Steel Group Co., Ltd., China). The anode medium contained 0.1 M phosphate buffer solution (PBS, pH 7.0), 0.39 g/L NH₄HCO₃, 10 mL/L mineral solution (Lovley and Phillips, 1988) and 10 mL/L vitamin solution (Lovley and Phillips, 1988). Acetate (30 mM) was added into the anode chamber as electron donor and replenished when it was depleted. The cathode solution contained 0.1 M PBS (pH, 7.0) and was bubbled with air at a rate of 200 mL/min. During the anode biofilm formation process, approximately the same magnitude of voltage was produced in the five reactors. When a stable voltage was produced for three continuous cycles, the five reactors were stopped and identical mixed anode biofilms were supposed to be obtained. One of the five reactors was randomly selected for power density measurement and the result revealed that the maximum power density was obtained under a 180 ohm external resistance.

Three of the four remaining reactors were employed for coking wastewater (CW, collected from the effluent of biological treatment unit, TOC = 28.3 mg/L) treatment by the cathodic bioelectro-Fenton process (Feng et al., 2010a,b). The solution in the cathode chamber was replaced with CW containing 0.1 M PBS (pH, 7.0) and 1 g γ-FeOOH (homemade according to Li et al., 2007). These reactors were operated under different modes, namely, MFC (180 ohm external resistance), MSC (short circuit, 0 V was supplied by an electrochemical workstation in the two-electrode mode) and MEC (0.3 V was supplied with an electrochemical workstation in the two-electrode mode). During the entire operation process, the catholyte pH was kept at 7.0. For investigating the anode characteristic in different BESs, representative anode potentials were recorded when MSC and MEC reached current peaks. The three reactors were continuously operated for 40 h because the cathodic TOC showed little change in the later period. Then, catholyte samples were taken for TOC measurement to determine the treatment efficiency.

In the meantime of CW treatment process, the last one of the five reactors was employed for the anode-biofilm CV measurements in the presence and absence of acetate, respectively. The CV of anodic planktonic bacteria was also conducted with a fresh graphite felt. In addition, this anode biofilm was also utilized for EIS measurement for investigating the charge transfer resistance (Rct) in three BES conditions. It should be noted that some necessary control tests were also conducted and this entire study was repeated in a biochemical incubator at 30 °C.

2.3. Measurements and analysis

The voltage output in MFC was recorded by a data collector (AD8223, Analog Devices, USA), and the current generation in MSC and MEC was recorded by an electrochemical potentiostat (CHI 1000a, Chenhua Instrument, China). CV and EIS of the anode biofilm were performed using the CHI 660c electrochemical potentiostat (CHI 660c, Chenhua Instrument, China). The EIS measurements were recorded in the frequency range from 100 kHz to 10 mHz with a typical three-electrode mode, and the EIS result was fitted by ZSimpWin software to determine the Rct and exchange current. All the potentials recorded in the following sections were reported vs. saturated calomel electrode (SCE). Wastewater sample was analyzed by a TOC analyzer (TOC-V CPN, Shimadzu, Japan).

2.4. Calculations

The current generation in MFC was calculated according to Ohm's law as previously described (Feng et al., 2010a,b).

The exchange current I0 (A) is given by Eq. (1) (Bard and Faulkner, 2001),

\[
I_0 = \frac{R_T T}{nF R_{ct}}
\]

where Rct is the gas constant, T(k) is the temperature, n = 1 is the number of electron transferring from one bioelectroactive species molecule to electrode, F is Faraday’s constant, and Rct (ohm) is the charge transfer resistance.

3. Results and discussion

3.1. Current generation by the anode biofilm under different BES operational modes

After the biofilm formation process, ARB were successfully attached onto the anode surface according to the scanning electron microscope images (data not shown). The current generation is a representative for reflecting the anode-biofilm ET behavior in dif-
different BES operational modes. Fig. 1 shows that the current was produced with different magnitudes in MFC, MSC and MEC during the 40 h. Obviously, the current generation in MFC maintained a stable value of approximately 0.92 mA during the entire operation process. Nevertheless, the current generation both in MSC and MEC were much higher than that obtained in MFC. Moreover, the current peak produced by MEC (8.21 mA) was also twice higher than that produced by MSC (4.15 mA). In all, the great differences (areas marked with #1, #2 and #3 in Fig. 1) in current demonstrated that the ET rate of the anode biofilm largely depended on the BES operational mode. In addition, the control experiment (data not shown) demonstrated that the current generation contributed by pure electrochemical electrolysis can be neglected.

3.2. Bioelectroactive species in the anode biofilm investigated by CV

The great variation in current generation in different BESs can be related to the different electrochemical behaviors of the anode biofilm. Fig. 2b presents a sigmoidal shape CV of the mixed anode biofilm which was measured when the acetate was replenished. The current in the oxidative sweep stage represented the ET from the anode biofilm to the anode surface. When the potential was more negative than −0.505 V (e.g., −0.600 to −0.505 V), the absolute current value appeared extremely low and exhibited a lag phase. However, the oxidative current increased rapidly and exhibited a growth phase when the potential increased from −0.505 to −0.165 V. At last, when the potential was more positive than −0.165 V (e.g., −0.165 to +0.200 V), the oxidative current maintained a high and stable value and exhibited a saturation phase. This phenomenon was probably resulted by the bioelectroactive species which feature different formal potentials (Fricke et al., 2008).

The existence of bioelectroactive species in the mixed anode biofilm in this study is not clear. Thus, CV of the anode biofilm was conducted in the acetate-depleted solution to investigate the possible bioelectroactive species functioning in the current generation process (Fricke et al., 2008). Fig. 2c shows that there were several redox couples with an onset oxidative potential of −0.505 V in the CV. However, no distinct redox peaks were observed in the control test measured with a fresh graphite felt in the same acetate-depleted solution. This indicated that the redox couples were contributed by the bioelectroactive species on the mixed anode biofilm. Among these redox peaks, two major redox couples with formal potentials of −0.402 V (f2) and −0.345 V (f3) were distinctly observed. Furthermore, the first derivative (inset of Fig. 2c) of the voltammetric curve (−0.50 to −0.42 V in the oxidative sweep stage) revealed a less evolved anodic peak at −0.435 V, which corresponded to a formal potential of −0.473 V (f1). These results suggested that bioelectroactive species at least three different formal potentials were present in the mixed anode biofilm. Based on the relationship between these formal potentials and potential areas in lag (growth and saturation) phase, it can be considered that no or very few bioelectroactive species functioned as electron mediators in the lag phase. Nevertheless, bioelectroactive species began to function and promptly generated current (Fig. 2b) when electrode potential increased in the growth
phase. In the saturation phase, no more (new) species served as ET mediators and thus the current generation maintains relatively stable. This inference can be further demonstrated by the first derivative (data not shown) of the oxidative sweep stage of Fig. 2b; three extreme points appeared in the first derivative indicating the roles of \( f_1 \), \( f_2 \), and \( f_3 \) in promoting the ET process. On the whole, these results suggested that the bioelectroactive species on the mixed biofilm indeed functioned in the current generation process.

3.3. Different amounts of bioelectroactive species functioning in BESs

Based on the different formal potentials of the bioelectroactive species, it can be supposed that not all of these species function in every BES. Apparently, the amount of available bioelectroactive species would be significantly influenced by the anode potential during the BES operation process. Fig. 2a shows that the anode potential in BESs increased in the sequence of MFC (−0.468 V) < MSC (−0.396 V) < MEC (−0.278 V).

Fig. 3 shows the electronic potential energy (expressed as potential) in different electron carriers during the ET process (Wang et al., 2009; Finkelstein et al., 2006). \( E_1 \) is the decay of potential energy after a complex respiratory chain and electron-transfer chain in ARB cell; \( E_2 \) reflects the driving force of ET from bioelectroactive species to the anode; \( E_3 \) is the electric energy production/consumption associated with the difference between the anode potential and the cathode potential; and \( E_4 \) reflects the driving force of oxygen reduction with electrons. It is noticeable that \( E_2 \) is the key link which directly influences the anode-biofilm ET behavior. Based on the Gibbs free energy \( \Delta G = -nF\Delta E \), where \( n \) is the number of ET, \( F \) is Faraday’s constant, and \( \Delta E \) is the potential difference between the oxidant (e.g., anode) and the reductant (e.g., bioelectroactive species), it can be supposed that the value of \( E_2 \) (corresponding to \( \Delta E \)) determines the ET behavior in BES. When \( E_2 < 0 (\Delta G > 0) \), which means the anode potential is more negative than bioelectroactive species, the ET cannot happen according to thermodynamics. In contrast, when \( E_2 > 0 (\Delta G < 0) \), theoretically, bioelectroactive species will function as mediators shuttling electron from the biofilm to the anode.

The variation in current production among different BESs can be understood by the anode-biofilm ET behavior according to Gibbs free energy. As shown in Fig. 2a, all anode potentials in the three operational modes were more positive than the onset oxidative potential and \( f_1 \), indicating that biooxidation can readily occur. However, the bioelectroactive species whose formal potentials equal to \( f_2 \) and \( f_3 \) are not likely to be oxidized in MFC due to the relationship of \( f_3 > f_2 > -0.468 \text{ V} \). According to the same principle, species with the formal potential of \( f_3 \) cannot be oxidized in MSC while probably shuttle electrons to the anode of MEC because of \(-0.278 \text{ V} > f_3 > -0.396 \text{ V}\). Therefore, the amount of available bioelectroactive species varied greatly in different BESs. This result can be further demonstrated by means of the impedance in ET process.

\( R_{ct} \) is known to directly reflect the impedance of ET from the anode biofilm to the anode surface. To investigate the anode-biofilm \( R_{ct} \) in MFC, MSC and MEC, EIS was conducted intentionally at initial potentials corresponded to their anode potentials, respectively. Fig. 4 shows the EIS results (in the form of Nyquist plot) and corresponding curves fitted by the relevant equivalent circuit (Feng et al., 2010a). The equivalent circuit consists of an ohmic resistance \( R_{ohm} \), a \( R_{ct} \) and a double layer constant phase element (CPE). In general, the high frequency intercept with the x-axis denotes the \( R_{ohm} \), and the diameter of Nyquist arc at the middle frequency range indicates the magnitude of \( R_{ct} \). The values (Table 1) of these resistances were obtained by equivalent circuit fitting. Clear visible was that all the EIS had an approximately equal \( R_{ohm} \) because of the identical nature of electrode material and biofilm (e.g., biomass). However, Table 1 also reveals that the \( R_{ct} \) measured at −0.278 V had the smallest resistance (15.9 ohm), which was only 1/7 and 1/2 of those measured at −0.468 and −0.396 V, respectively. This result suggested that the ET impedance of anode biofilm decreased in the order of MFC, MSC and MEC. Based on the \( R_{ct} \) value, exchange current was calculated and Table 1 shows that it decreased in the opposite order of \( R_{ct} \). According to the concept of exchange current, it is easy to understand that such variation is attributed to the different amounts of activated species accessible to the electrode. Therefore, the EIS results demonstrated that the different amounts of available bioelectroactive species contributed to the different anode-biofilm ET behaviors in MFC, MSC and MEC.

3.4. Advanced CW treatment under different BES operational modes

Generally, the efficiency of wastewater treatment is significantly determined by the current generation in BES. For instance,
the difference of anodic COD removal among MFC, MSC and MEC can be visually reflected by the colorful areas marked with #1, #2 and #3 (Fig. 1), which also revealed that every BES device was able to remove anodic COD. However, till now, the feasibility of advanced wastewater treatment toward non-degradable compounds by BES is not clear. Results of CW treatment revealed that MFC and MSC enabled TOC removal efficiency of 5.1 ± 1.7% and 18.2 ± 3.2%, respectively (data not shown) after the bio-electro-Fenton process. In contrast, MEC achieved the maximum of 53.9 ± 4.2%, which was almost eleven and three times those of MFC and MSC, respectively. Furthermore, energy analysis revealed that only 0.452 kWh/d m² was consumed by MEC, which was much smaller (even negligible) compared with the traditional electrolysis cell (Cheng et al., 1997). This suggested that MEC might be the only feasible device for advanced wastewater treatment. In addition, the control test (data not shown, operated under the same condition of MEC but without aeration) suggested that the direct electrochemical reduction of CW can be neglected. Apparently, the current generation in BES by the anode biofilm is an important factor resulting in such great differences. In the MFC and MSC mode, the electrons cannot further be transported out after accumulating in the bioelectroactive species whose formal potentials were higher than the anode, resulting in a limitation for current generation. On the other hand, this phenomenon did not occur in MEC, which ensured a relatively higher current and higher cathodic H₂O₂ yield (Rozendal et al., 2009). From a chemical reaction view, the efficiency of advanced wastewater treatment is greatly determined by the oxidant concentration (e.g., H₂O₂) because the reductant concentration (e.g., COD or TOC) is very low. Therefore, according to the results in this study, operational mode should be carefully selected with respect to the future BES application.

4. Conclusion

This study investigated the anode-biofilm ET behavior and advanced wastewater treatment under different BES operational modes. Different amounts of available bioelectroactive species functioned as ET mediators in MFC, MSC and MEC, respectively. Such differences resulted in a significant variation of current generation, which increased in the sequence of MFC, MSC and MEC. Due to the superior capacity of current generation, MEC was supposed to be the only feasible operational mode for advanced wastewater treatment. According to the anode-biofilm ET behavior and wastewater treatment efficiency, operational mode should be especially considered with respect to the future application of the BES technology.

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Table 1
Charge transfer resistance and exchange current of anode biofilm.

<table>
<thead>
<tr>
<th>Sample measured potential (V)</th>
<th>Rθthin (ohm)</th>
<th>Rθ (ohm)</th>
<th>Exchange current (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−0.468</td>
<td>9.4</td>
<td>110.9</td>
<td>2.4 × 10⁻⁴</td>
</tr>
<tr>
<td>−0.396</td>
<td>9.2</td>
<td>31.6</td>
<td>8.3 × 10⁻⁴</td>
</tr>
<tr>
<td>−0.278</td>
<td>8.9</td>
<td>15.9</td>
<td>1.6 × 10⁻³</td>
</tr>
</tbody>
</table>

References