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Flat microliter membrane-based microbial fuel cell as "on-line sticker sensor" for self-supported *in situ* monitoring of wastewater shocks

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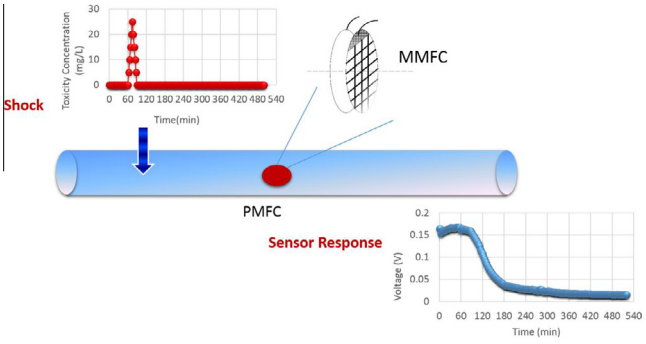
HIGHLIGHTS

- Flat microliter MMFC was developed as "on line sticker sensor".
- Hydrophilic and microporous membrane shortened the acclimation duration.
- MMFC sensors responded well to Cr^{6+} and Ni^{2+} shocks in wastewater.
- OCP of MMFC sensors well reflected shock types and concentrations.
- The voltage of the MMFC was clearly correlated with shock concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Novel flat membrane-based microbial fuel cell (MMFC) sensors were developed by compacting two filter membranes coated with carbon ink. High micro-porosity and hydrophilicity of membranes offered the distinct advantages of short acclimation period (couple hours), simple compact configuration with microliter size, and high sensitivity and stability. MMFC sensors were examined at two toxic shocks (chromium and nickel) in a batch-mode test chamber, and rapidly responded to shock types and concentrations. The variation of voltage output was correlated with open circuit potential (OCP). Filter membranes facilitated bacterial attachment and shortened acclimation. The MMFC sensors showed good reusability and recovered several days after toxic shocks. The robustness of MMFC sensors was validated through 1-month tests. The stability of sensor signals was examined with coefficient of variance (CV) statistical analysis. The flat microliter MMFC has a great potential as "on-line sticker sensor" for real time *in situ* monitoring of wastewater quality.

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1. Introduction

Wastewater treatment plants (WWTPs) have been designed and operated based on the average concentrations of contaminants. Short-term and long-term shocks (e.g. organic compounds and heavy metals) in wastewater disturb the stability of WWTPs. Real-time wastewater shock sensors are critical to provide effective precaution strategies and minimize shock impacts. Diverse biosensors (e.g. biological oxygen demand, ammonia, and heavy metal) have been developed to monitor the contaminants in wastewater. However, the biosensor performance is directly depended on the coated enzymes and microorganisms, which have inherent problems, such as short lifetime (Okochi et al., 2004;

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Curtis et al., 2009; Woznica et al., 2010), long respond time (Jiang, 2008), narrow specificity for chemicals (Curtis et al., 2009; Ikebukuro et al., 1996; Neufeld et al., 2006; Okochi et al., 2004), and the need for external power sources (Ikebukuro et al., 1996; König et al., 1998; Kumlanghan et al., 2008).

A novel bioelectrochemical device, microbial fuel cell (MFC), has been studied to convert wastewater to electricity by electrogenic bacteria (Liu et al., 2005; Oh and Logan, 2005; Pant et al., 2012; Li et al., 2014b). MFC possesses three unique features as wastewater sensors. First, wastewater shocks can cause immediate jump or drop of the voltage output of MFCs, which can be used as the real-time shock indicator. Second, with the electricity generated from wastewater to support its operation, MFCs are self-sustainable without external power supply. Third, MFCs utilize the microorganisms and organic substrates in wastewater without the need of coating external enzymes and bacteria, which simplifies its setup and prolongs the lifetime. MFC sensors have been developed for biological oxygen demand (BOD) (Chang et al., 2005), chemical oxygen demand (COD) (Wang et al., 2014), organic substances (Kumlanghan et al., 2007), and toxins (Liu et al., 2014) in wastewater. However, existing MFC sensors still utilize traditional MFC configurations (e.g. tubular bioreactor, single chamber, and cube shape) (Chang et al., 2005; Wang et al., 2014; Kumlanghan et al., 2007; Liu et al., 2014), which poses difficulties for real-time shock monitoring. These MFC sensors have the volume of 20–150 mL with inlets and outlets (Chang et al., 2005; Wang et al., 2014; Kumlanghan et al., 2007; Liu et al., 2014), which are the operational systems by themselves, and make it difficult for direct installation on wastewater facilities. In addition, the voltage output of MFC sensors is closely associated with open circuit potential (OCP) and inner resistance (R_{in}) (Logan et al., 2006), and keeping stable R_{in} is critical for reducing the possibility of fault signals of MFC sensors. But large volume (normally 20–150 mL) of wastewater contained the existing MFC sensors may cause unstable R_{in}, and increase the possibility of fault signals. Moreover, these MFC sensors need at least 1–2 weeks to acclimate electrogenic bacteria, meaning that they have to be inoculated long time before the occurrence of shocks, which is unrealistic for monitoring unexpected wastewater shocks.

A new type paper-based MFC, which packed carbon cloth and paper-based proton exchange membrane (PEM) together, could solve the problem of MFC sensors (Fraiwan et al., 2013a,b; Nguyen et al., 2014). The paper-MFC utilized both sides of carbon cloth as anode and cathode, which substantially reduced the MFC volume from milliliter (mL) to microliter (μL). However, this paper-MFC is still impractical for on line monitoring. Ferricyanide was used as the electron acceptor in cathode, which is not suitable for real-world application due to its toxicity and high cost. An additional hydrophilic paper reservoir (size: 4 cm × 3 cm) was used to shorten the acclimation time (Fraiwan et al., 2013a,b; Nguyen et al., 2014), which made the paper-MFC configuration complicated and difficult for direct installation in WWTPs. Most importantly, low mechanical strength of paper reservoir and water leakage of carbon cloth posed obstacles for *in situ* monitoring.

The objective of this study was to develop a simple compact membrane MFC (MMFC) sensor by stacking two flat filter membranes without the PEM and paper reservoir. The flat MMFC sensors possess four major breakthroughs over traditional MFC sensors (Chang et al., 2005; Kumlanghan et al., 2007; Wang et al., 2014; Liu et al., 2014) and paper-MFCs (Fraiwan et al., 2013a,b; Nguyen et al., 2014). MMFC sensors minimize the size to microliter (μL), which is expected to substantially reduce the variability of R_{in} and the possibility of fault signals. In addition, oxygen in the air is used as the electron acceptor in the MMFC sensors, which simplifies MMFC configuration. The unique flat structure of MMFCs makes the direct installation on wastewater facilities possible and serves as an "on line sticker sensor" for real time *in situ* wastewater quality monitoring. Moreover, microporous membranes could solve water leakage and enhance mechanical strength. High hydrophilicity of membranes is expected to facilitate bacterial adhesion and shorten acclimation time. Finally, the flat MMFC is studied as the shock sensor, while the paper-MFC was still targeted as power generation.

There were five tasks in this study. First, the flat MMFC sensors were installed as a sticker in a batch mode test chamber to examine its acclimation duration and voltage output. Second, the responses of the MMFC sensors to two toxic shocks (chromium and nickel) were determined at different concentrations. Third, OCP of the MMFC sensors was measured and correlated with the sensor performance. Fourth, the biofilm morphology and the contact angles of membranes were compared with carbon cloth (common anode materials for MFCs) to elucidate the advantage of MMFC sensors. Finally, the long-term robustness of flat MMFC sensors was examined through 1-month operation. The stability of sensor signals was validated using coefficient of variance statistical analysis.

2. Methods

2.1. Fabrication of flat MMFC sensor

The anode and cathode of the flat MMFC sensor were fabricated on filter membranes (Mixed Cellulose Ester Membranes, diameter: 4.7 cm, pore size: 0.22 μm, Millipore) (Fig. 1a). Specifically, multiple lines (thickness: <1 mm) of carbon ink (Microcircuit Material, DuPont) were painted on the filter membranes using a brush to effectively transfer electrons generated by electrogenic bacteria growing on membranes. The anode membrane was coated with plain carbon ink, while the cathode membrane was coated with plain carbon ink plus a top layer of platinum (Pt as the catalyst, loading: 0.5 mg/cm^2) (Nguyen et al., 2014). The resistance of stacked membranes was measured across the membrane (the longest distance) using a multimeter (Fig. 1a). Before the assembly, the anode membrane was soaked into a single chamber MFC (SCMFC volume: 300 mL) for 3 h to acclimate microorganisms on the membrane surface. The SCMFC had been operated for several weeks treating wastewater taken from the influent of the University of Connecticut WWTP (COD: 250–350 mg L^{-1} and BOD: 100–300 mg L^{-1}). The microorganisms acclimated on the anode were intrinsic in wastewater, so that MMFCs would not be a pollution source for wastewater. Next, the anode and cathode membranes were stacked together with the sides of coated carbon ink facing outside (Fig. 1b). Copper wires were bonded on the top bulk area of membranes for multimeter connection. To elucidate the importance of bacterial attachment for MMFC sensors, a control membrane was soaked into deionized water for 3 h and then assembled with a cathode membrane. The voltage of this blank control was compared with MMFC sensors.

2.2. Test chamber setup and shock tests

A test chamber made of plexiglass was used to simulate a wastewater facility (Fig. 1c). The MMFC sensor (volume: <200 μL, diameter: 5 cm) was inserted firmly into one side of the test chamber as an "on line sticker", with the cathode membrane facing to air (oxygen as the electron acceptor) and the anode membrane facing to wastewater in the container (substrates in wastewater as electron generator). The surface structure of the MMFC sensor was observed after 1-month operational period to examine the robustness and mechanical strength of membranes. Throughout the test period, the chamber was full of anoxic wastewater, with the redox potential (ORP) below −350 mV.

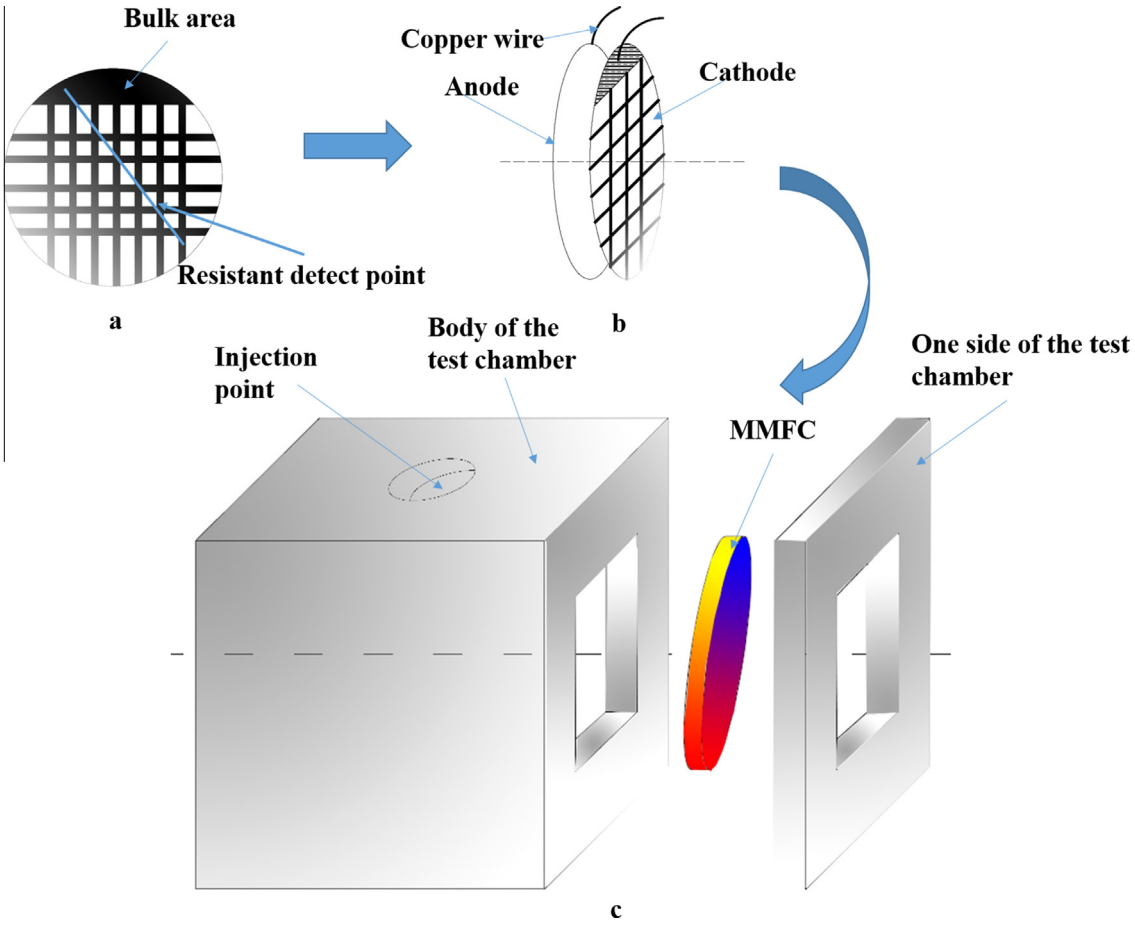

Fig. 1. Diagram of the anode/cathode filter membrane (a), the assembly of a MMFC sensor (b), and the MMFC sensor installed as the "on line sticker" of a batch-mode test chamber (c).

Two types of toxic metal shocks were injected to the test chamber using a syringe through the rubber septum (diameter: 10 mm) (Fig. 1c). Chromium (Cr^{6+}) is a well-known toxic metal with the limitation of 0.1 mg L^{-1} in drinking water (EPA 816-F-09-0004), and high Cr^{6+} concentration (>10–20 mg L^{-1}) occurs in wastewaters from electroplating and chrome tanning (Monser and Adhoum, 2002; Gupta et al., 2001). The Cr^{6+} shocks were examined at three concentrations: 5 mg L^{-1} as the upper-end Cr concentration in wastewater (the control test), 10 mg L^{-1} and 20 mg L^{-1} as the shocks in wastewater influent, which was achieved by injecting 150 μL, 300 μL, and 600 μL K_2CrO_4 solution (1000 mg L^{-1} Cr^{6+}) into the chamber individually. The dilution effect was ignored since the injection volume (150–600 μL) only counted for 1% of the chamber volume (30 mL). Cr^{6+} concentrations in the container were measured using the colorimetric standard method (Varian Cary 50 Bio UV–VIS spectrophotometer) (Li et al., 2014a) at 2 min, 30 min, 2 h, and 20 h after the Cr^{6+} injection (20 mg L^{-1}). The nickel toxicity (Ni^{2+}) is not as acute as Cr^{6+} (Liu et al., 2014; Stein et al., 2012; Qin et al., 2006), although the limitation of 0.1 mg L^{-1} in drinking water is same as Cr (EPA 816-F-09-0004). Previous MFC sensor study showed that Ni (30 mg L^{-1}) caused a sudden drop in the voltage output (Stein et al., 2012). Ni^{2+} concentration could be 1000 mg L^{-1} in the plating wastewater (Qin et al., 2006). The Ni^{2+} shocks were examined at three concentration: 5 mg L^{-1} as the upper-end Ni concentration in wastewater (the control test), 20 mg L^{-1} and 50 mg L^{-1} as the shock in wastewater influent, which was achieved by injecting 50 μL, 200 μL, and 500 μL $NiSO_4{\cdot}6H_2O$ solution (3000 mg L^{-1} Ni^{2+}) into the test chamber individually. All shock tests were duplicated.

2.3. Internal resistance of the MMFC sensor

The external resistance ($R_{ext.}$) was 1466 Ω and the voltage output was recorded using a Keithly 2700 data logging system for 1-month operation period (Liu et al., 2014). The voltages over a series of ($R_{ext.}$: 36–21,090 Ω) of the MMFC sensor were recorded using a digital multimeter (Watson and Logan, 2011) to get the power density curve. The inner resistance (R_{in}) was determined based on the maximum value on the curve. The R_{in} measurement was duplicated.

2.4. Stability analysis of MMFC voltage output

Two datasets were taken during a 5-h period to examine the stability of voltage output and distinguish sensor signals from noises. One dataset was taken 5 h before shock injection (termed as V_{bs}), and the other was 2.5 h before shock injection and 2.5 h after shock injection (termed as V_{bs+as}). Each dataset consisted of 10 data points (with one data point per half hr). Statistical analysis of these datasets was performed using Minitab 17 with coefficient of variance (CV) analysis.

2.5. Biofilm formation on the anode membrane

Biofilms growing on the anode membrane of the MMFC sensor after 3 h and 1-month period were observed and compared with carbon cloth (Fuel Cell Earth LLC, MA) (common anode material used in MFCs) (Li et al., 2014a). Scanning electron microscope

Table 1
OCP value of the MMFC sensor before and after Cr^{6+} and Ni^{2+} shock tests.

Shock type	After setup	No shock	5 mg L^{-1} Cr^{6+}	10 mg L^{-1} Cr^{6+}	20 mg L^{-1} Cr^{6+}	5 mg L^{-1} Ni^{2+}	20 mg L^{-1} Ni^{2+}	50 mg L^{-1} Ni^{2+}
OCP (mV)	512.5 ± 7.5	378 ± 5	343 ± 3	66 ± 1	67 ± 1	367 ± 2.5	313 ± 3	282 ± 2

(SEM) observation (Model: Joel6335F) was conducted as previously described (Santoro et al., 2011).

2.6. Contact angle measurements

Bacterial attachment on a solid surface is correlated with the surface hydrophilicity (Fraiwan et al., 2013 a; Li and Logan, 2003). The static contact angles of the clean membrane and clean carbon cloth were examined in duplicate tests using a CAM 101 optical surface tension meter (KSV Instrument Inc.) (Andresen et al., 2006).

3. Results and discussion

3.1. Acclimation and stabilization of the MMFC sensors

After the anode filter membrane soaked into a SCMFC solution for 3 h, it was assembled with the cathode filter membrane as a MMFC sensor and then installed into the test chamber. The MMFC sensor instantaneously had the OCP of 512.5 ± 7.5 mV and stabilized at 378 ± 5 mV, while the OCP of the control MMFC (with the anode membranes being soaked into deionized water before assembly) was only 90 ± 10 mV (Table 1). Because the OCP value is associated with the bacterial electrogenic activity (Liu et al., 2014; Santoro et al., 2012), this comparison showed that sufficient bacteria attached to the anode membrane within 3 h soaking in the SCMFC solution and started the electron transfer instantaneously after being exposed to wastewater in the test chamber. In contrast, the control anode membrane with blank surface (without bacterial adhesion) had low electron transfer capability exhibited by low OCP. Traditional MFCs with carbon cloth or activated carbon as the anode materials took at least 3–15 days to acclimate bacterial growth and achieve good voltage (OCP > 300 mV) (Chang et al., 2005; Wang et al., 2014; Kumlanghan et al., 2007; Liu et al., 2014; Li et al., 2014a).

The short acclimation time of the MMFC sensors was caused by the rapid absorption of the analytes (e.g. wastewater and bacterial cells) and the acceleration of bacterial attachment to the membrane surface (Fraiwan et al., 2013a). The immobilization efficiency depends on the hydrophilic and hydrophobic proprieties, pore size and shape. (Yang et al., 2012). This mechanism of high hydrophilic membrane was verified by lower contact angle (56°, hydrophilic) of filter membrane than carbon cloth (133°, hydrophobic) (Fig. S1). The pores (diameter: 0.22 μm) of the filter membrane (Fig. S2a) are much smaller than the carbon cloth fiber (>10–15 μm) (Fig. S2b), and possess high surface area. Previous studies had found that filter membrane with high surface areas had high loadings of bioentities (Kolenbrander et al., 2010; Yang et al., 2012), which was confirmed by good bacterial attachment on membranes after 3-h soaking in wastewater (Fig. S2c). Because biofilms on anode surface were formed by bacterial cells sticking to each other (Costerton et al., 1978), the microporous matrix of membrane greatly shortened the distance between cells and led to a much faster acclimation (Fig. S2d) than carbon cloth (Fig. S2e). The unique properties of membranes (hydrophilic and microporous) simplified the operation and avoided the long incubation time required for the MFC sensors with traditional configurations and electrode materials.

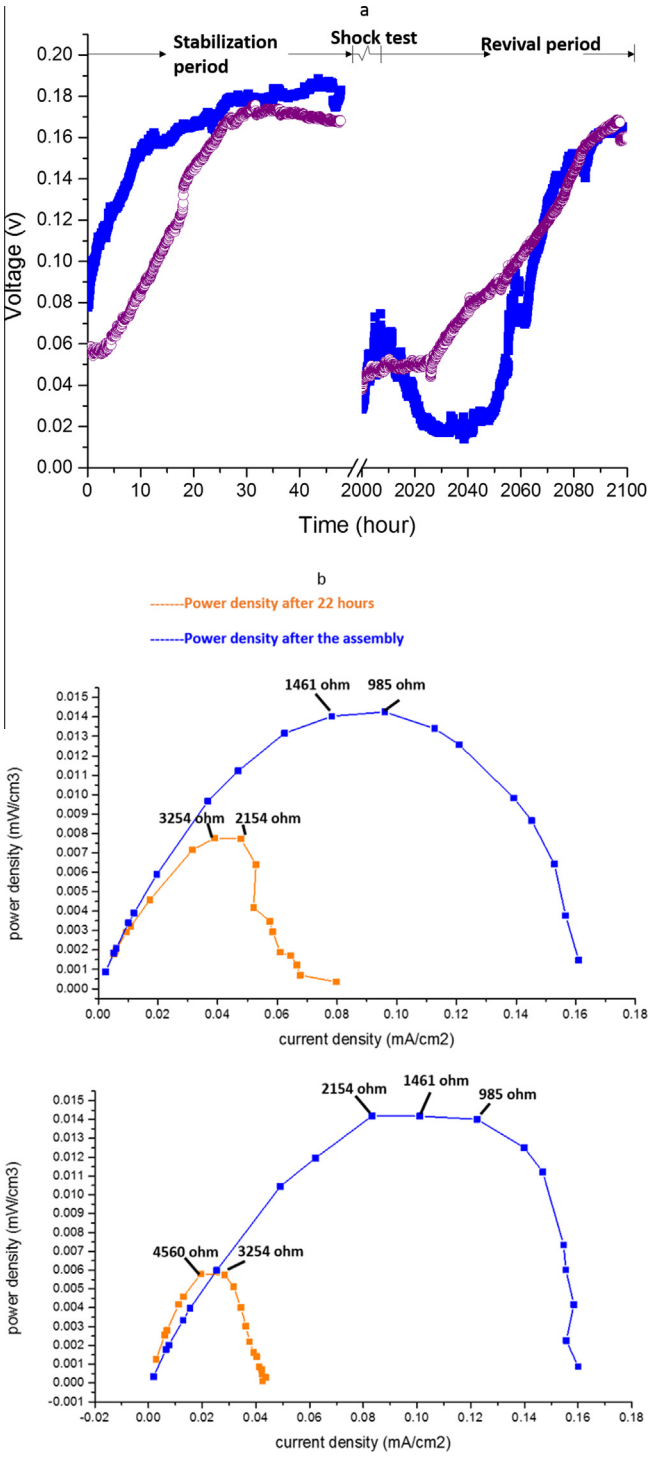

Fig. 2. Stabilization of the MMFC sensor after installation in the container and the revival tests after 10 mg L^{-1} Cr^{6+} shock tests (a), power density curves of the MMFC sensor right after assembly and 1 day in the container (b). (Both tests were duplicated.)

The voltage output of the MMFC sensor started at 0.08 V and stabilized at 0.18 V after 25 h installation in the test chamber fed

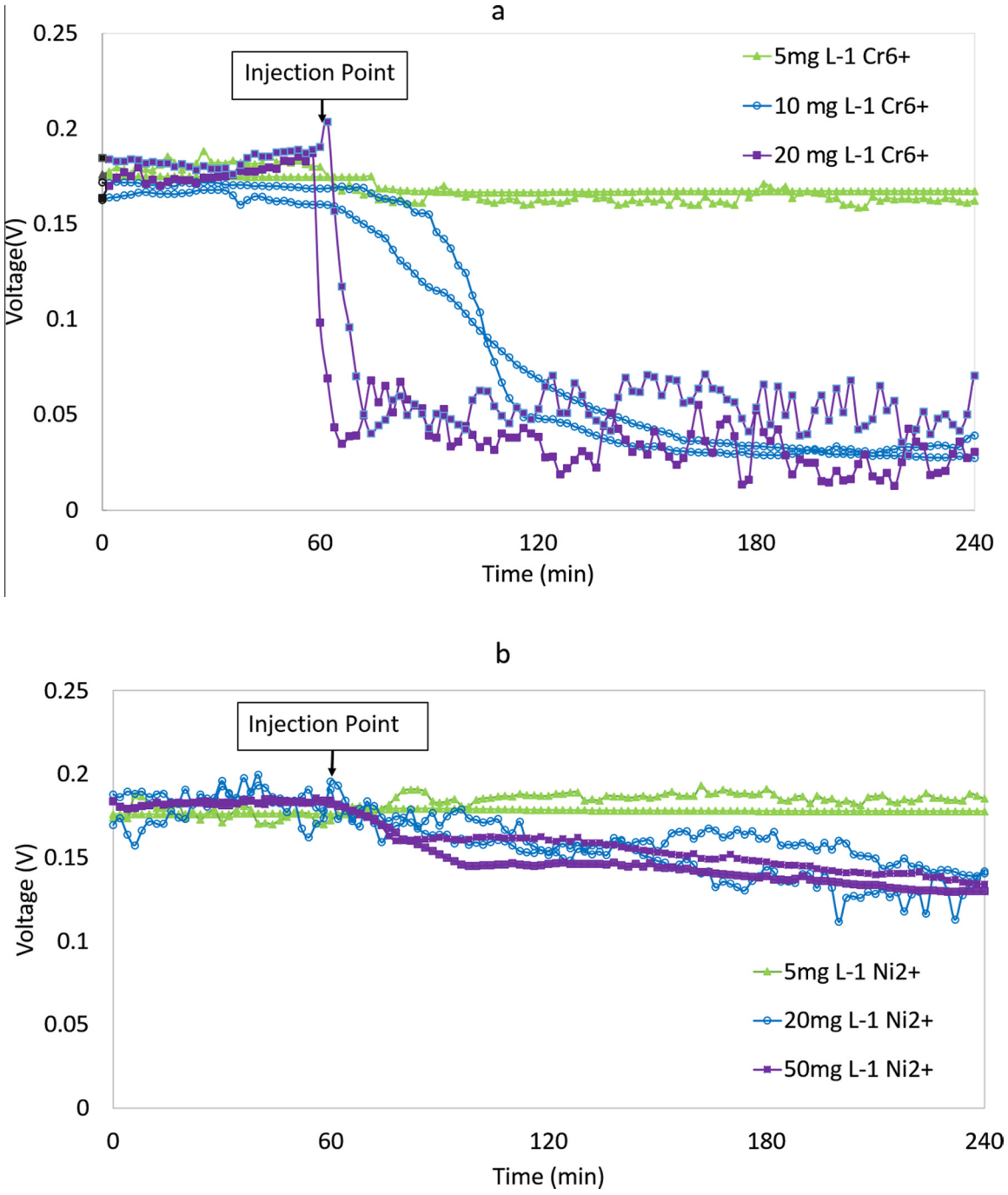

Fig. 3. Voltage responses of the MMFC sensor to 5, 10, and 20 mg L^{-1} Cr^{6+} (a) and 5, 20, and 50 mg L^{-1} Ni^{2+} (b) (duplicate tests for each shock).

with fresh wastewater (Fig. 2a), while the OCP decreased from 512.5 ± 7.5 mV to 378 ± 5 mV (Table 1), indicating that the activity of microorganisms slightly decreased and reached a stable status. The R_{in} dropped with time (Fig. 2b), which was corresponded with the increase in voltage output. The R_{in} was 956–1140 Ω, which was higher than other MFC sensors (~500 Ω) (Liu et al., 2014). The resistances of anode and cathode membranes were 330 ± 8 Ω and 224 ± 2 Ω, respectively, indicating that there were other factors (e.g. the space between membranes) affecting the R_{in} (Mook et al., 2013). Two potential approaches could be adapted to lower R_{in}. First, the carbon ink layer on membranes could be automatically coated using a printer rather than manually coating in this study, which will produce an evenly-distributed carbon layer. Second, the packing process of anode/cathode membranes of the flat MMFC sensor (Fig. 1b) could be conducted using a pressing machine to minimize the space between the anode and cathode membranes.

3.2. Sensitivity of the MMFC shock sensors

The MMFC sensors were examined at two types of toxic shocks (Cr^{6+} and Ni^{2+}). The voltage output of the MMFC stabilized at 0.18 V before the shock injection, and slightly dropped to 0.16 V within 180 min after 5 mg L^{-1} Cr^{6+} shock (Fig. 3a). Previous study using a cube MFC sensor (volume: 30 mL) showed that the voltage dropped from 0.12 V to 0.07 V within 60 min of the Cr^{6+} shock (2 mg L^{-1}) (Liu et al., 2014). The MMFC exhibited a higher tolerance for Cr^{6+} than traditional MFC sensors, which might be caused

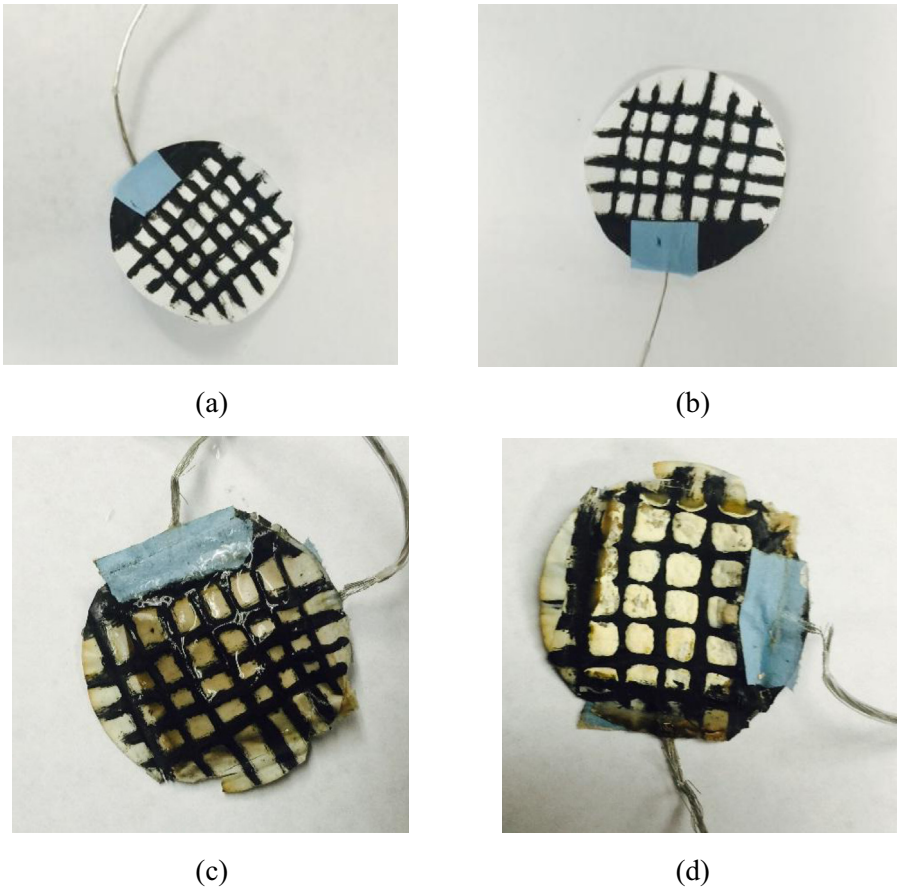

Fig. 4. The pictures of the clean anode (filter membrane) and the clean cathode (filter membrane) before assembly (a and b), the anode and cathode after 1-month period (c and d).

Table 2
Stability analysis of the voltage output of the MMFC sensor 5 h before and 2.5 h before and after shock injection (A and B are duplicated tests).

Variable	Mean value of voltage	StDev*	Variance of voltage	CoefVar*
A				
Voltage 5 h before shock (V_{bs})	0.16862	0.000736	0.000001	0.44
Voltage 2.5 h before shock and 2.5 h after shock (V_{bs+as})	0.09861	0.07039	0.00496	71.38
B				
Voltage 5 h before shock (V_{bs})	0.1832	0.00359	0.000013	1.96
Voltage 2.5 h before shock and 2.5 h after shock (V_{bs+as})	0.16105	0.03083	0.00095	19.14

* StDev and CoefVar stand for standard deviation and coefficient of variance, which are used to distinguish the shock signals from noises.

by the unique hydrophilic and microporous property of the membrane and good biofilm formation (Fig. S2d). Because shocks are the "exceptionally high concentrations (at least 5–10 times as normal concentrations)" occurring in wastewater, the flat curve of the MMFC voltage output at 5 mg L^{-1} Cr^{6+} (normally the high-end Cr concentration in wastewater) could be used as the control background for shocks to prevent the false signals. In contrast, the voltage rapidly dropped to 0.04 V 40 min after the 10 mg L^{-1} Cr^{6+} shock, and dropped to 0.05 mV 6 min after the 20 mg L^{-1} Cr^{6+} shock (Fig. 3a), demonstrating that the 20 mg L^{-1} Cr^{6+} shock caused faster and more dramatic voltage drop than the 10 mg L^{-1} Cr^{6+} shock. The MMFC sensor was capable of distinguishing the toxic concentration. The duplicate test followed the same trend. Previous study showed that the voltage dropped from 0.12 V to 0.04 V within 5 min of the Cr^{6+} shock (8 mg L^{-1}) (Liu et al., 2014). The reason for the lower sensitivity of the flat MMFC sensor in this study might be the higher R_{in} of the MMFC than that of the cube MFC sensor (R_{in}: 500 Ω) (Liu et al., 2014; Li et al., 2014a). The Cr^{6+} concentration (20 mg L^{-1}) in the test chamber was quite stable within 20-h period (Table S1), indicating that the voltage drop was caused by the inhibited electrogenic bacterial activity, rather than the electron consumption by Cr^{6+} oxidation. The OCP of the MMFC sensor dropped from 378 mV to 66 mV (Table 1), which confirmed the inhibited electrogenic bacterial activity.

The voltage responses of the MMFC sensor to the Ni^{2+} shocks were milder than Cr^{6+} shocks. The 5 mg L^{-1} Ni^{2+} shock caused no visible voltage drop within 180 min, which was verified by the similar OCP value before and after injection (378 mv vs. 367 mv)

(Table 1). The 20 mg L^{-1} Ni^{2+} shock only caused a slight voltage drop from 0.18 V to 0.15 V within 180 min (Fig. 3b), and 50 mg L^{-1} Ni^{2+} shock caused a faster voltage drop (0.18–0.15 V) within 45 min (Fig. 3b). The OCP measured at the end of the 50 mg L^{-1} Ni^{2+} tests was 282 ± 2 mV (Table 1), which was somehow lower than that of wastewater (~380 mV) but much higher than that of Cr^{6+} shocks (66 mV). The sharp voltage drop at Cr^{6+} shocks and slight drop at Ni^{2+} shocks implied that this difference in the voltage declining pattern of the MMFC sensor could be used to distinguish the shock types in wastewater.

The reusability is a critical factor for shock sensors. If all the bacteria are inactivated by toxic shocks without recovery, the biosensors have to be replaced each time after the occurrence of toxic shocks, which poses a potential problem for *in vivo* monitoring. The revival of the MMFC sensor was examined after the Cr^{6+} shock tests. Although the electrogenic bacterial activity was almost inhibited at the 10 mg L^{-1} and 20 mg L^{-1} Cr^{6+}, it gradually recovered to the original level (voltage: 0.14–0.18 V) 80 h after shocks (Fig. 2a, duplicated tests). During the revival period, the batch-mode test chamber was fed with wastewater containing 1000 mg L^{-1} sodium acetate and replenished daily. The results indicated that the MMFC shock sensor can be recovered after a short-term toxin shock albeit all the electrogenic bacterial activity was almost inhibited. Even though the Cr^{6+} shock in this study was much stronger than a previous study (8 mg L^{-1}, cube MFC sensor with a volume of 30 mL, Liu et al., 2014), the revival time of the MMFC sensor was similar to the reported value (about 3 days), which indicated a better reusability. In addition, because the MMFC sensor was examined in the batch-mode test chamber that replenished fresh wastewater once per day, rather than a continuous-mode system that receives fresh wastewater continuously to dilute the shocks over time, the actual revival time of MMFC sensors in WWTPs should be much shorter than the batch-mode test chamber used in this study (Lee et al., 2015). Finally, the MMFC was developed as "an on line sticker sensor" for wastewater shocks, which means the shocks will occur at exceptionally high concentration (e.g. 5–10 times as normal concentrations in wastewater) but at much less frequency (e.g. once per week/month/year). The revival time (2–3 days) of MMFCs needed would be sufficient for the recurrence of shocks in a given system. Considering the flat MMFC sensors' short acclimation time, easy fabrication and installation of on wastewater facilities, it is feasible to replace them if the bacterial revival time is too long.

The stability of MMFC sensor structure and sensor signals is critical for long-term application. The anode and cathode (filter membranes) were observed before (Fig. 4a and b) and after 1-month tests (Fig. 4c and d). Both membranes maintained high structure integrity without breaking and cracking, demonstrating the good mechanical strength for long-term *in vivo* monitoring. Compared with the anode with biofilms growing after 1-month operation (Fig. 4c), the cathode stayed dry (Fig. 4d), which confirmed there was no water leaking during the operation even though it is hydrophilic. No detectable water level drop was observed in the container during 1-month period, since the microporous matrix of membranes prevented the water leaking and pressure drop.

The coefficient of variance (CV) statistical analysis was conducted for the MMFC sensor. Duplicated tests showed that the StDev of V_{bs+as} (0.07039 and 0.03083) was about 10–100 times as that of V_{bs} (0.000736 and 0.00359) (Table 2), indicating that the voltage signal was quite stable before the shocks. The CoefVar of V_{bs+as} (71.38 and 19.14) was about 10–150 times as that of V_{bs} (0.44 and 1.96), indicating that the sensor signals after shock injection were predominant and much higher than the noise. The results clearly showed that the voltage output (V_{bs}) kept stable for a long time and the shock signals (V_{bs+as}) were predominant over noises, which effectively avoid the occurrence of false shock signals.

3.3. Significance of the flat MMFCs as "on line sticker sensors" for wastewater monitoring

By utilizing filter membrane as the electrode and biofilm support, and carbon ink as the electron collector, the flat MMFC reduces the sensor volume to μL, and maintains the simple compact configuration that can be directly installed on wastewater facilities as self-sustained "on line sticker sensors". No any reported biosensor or MFC sensor possesses this unique advantage. High hydrophilic and microporous of membranes induced excellent adsorption ability for electrogenic bacteria and shortened the acclimation time to several hours. One-month *in situ* tests of the MMFC sensors in a batch-mode chamber showed stable voltage output, good sensitivity to toxic metal shocks, good recovery from the shocks, and high mechanic strength. It should be noted that the due to the requirement of bacteria and organic substrates in the anode, MMFC sensors are suitable as the pre-screen tool for the presence or absence of wastewater shocks, not for contaminant concentration measurement (e.g. COD and metals) nor drinking water monitoring.

4. Conclusion

A novel flat membrane-based MFC (MMFC) sensor was developed in this study by directly stacking two filter membranes. The MMFC possessed distinct advantages over traditional MFC sensors and the reported paper-MFCs: short acclimation duration (couple hours), flat compact configuration, and microliter size, which provides a great potential as "on line sticker sensor" for wastewater quality monitoring. The batch-mode tests demonstrated that the MMFC sensors possessed a high sensitivity under the shocks of toxic metals (Cr^{6+} and Ni^{2+}), and exhibited a good reusability and a high stability of voltage signals.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.08.081.

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