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A batch-mode cube microbial fuel cell based "shock" biosensor for wastewater quality monitoring

Bingchuan Liu [a], Yu Lei [b], Baikun Li [a,*]

[a] Department of Civil and Environmental Engineering, University of Connecticut, 261 Glenbrook Road, Unit 3037, Storrs, CT 06269, USA
[b] Department of Chemical and Biomolecular Engineering, University of Connecticut, 191 Auditorium Road, Unit 3222, Storrs, CT 06269, USA

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ABSTRACT

A single chamber batch-mode cube microbial fuel cell (CMFC) was explored as a novel self-sustained biosensor for real-time monitoring the toxicity shocks (sudden change in toxins concentration) of representative toxic metals in wastewater influent. Four types of shocks, including chromium, iron, nitrate, and sodium acetate, were selected to represent the shocks of acute-toxic heavy metal, low-toxic metal, common nutrient, and organic contaminant in wastewater, respectively. Wastewater was used as the inoculum in CMFCs for anodic electrogenic bacteria that were fully acclimated within 3 days, which indicated that this self-powered sensor can be quickly adapted to wastewater. The results showed that the CMFC was able to distinguish shocks of toxins from non-toxins based on voltage signal changes. Anode open circuit potential (OCP) values were well correlated with the CMFC voltage changes, indicating that the voltage changes were mainly dependent on the activity of the electrogenic bacteria on the anode surfaces.

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1. Introduction

Municipal and industrial wastewater qualities vary over time and directly affect the performance of wastewater treatment plants (WWTPs). The concentrations of toxins (e.g. Chromium, Cyanide, and Nickel) could increase sharply and cause the severe interruption or irreversible damage of WWTPs, and the spike of certain organic/inorganic contaminants (e.g. nitrate, organic carbon substrates, and detergents) could cause fluctuation in the routine operations of WWTPs. Currently, most of chemical tests for wastewater quality monitoring are conducted off-site and cause the long time delay for WWTPs to recover after shocks. Developing on-line shock sensors becomes critical to monitor wastewater influent quality and to provide online early warning systems for timely precaution and solution.

In the past decade, various biosensors based on the interactions between microorganisms (or water plants) and organic/inorganic substrates have been developed to monitor parameters (e.g. biochemical oxygen demand (BOD), toxins, and chlorine) in water resources (e.g. natural water bodies, drinking water, wastewater, and estuary) (Ikebukuro et al., 1996; Konig et al., 1998; Campanella et al., 2001; Kim et al., 2003; Okochi et al., 2004; Neufeld et al., 2006; Kumlanghan et al., 2008; Curtis et al., 2009; Eltzov et al., 2009; Woznica et al., 2010; Oh et al., 2011; Dong et al., 2012; Jiang et al., 2004). However, four major problems of biosensors have hindered the real-time shock detection in water media. First, these biosensors utilize a certain type/group of microorganisms and can only detect specific known chemicals, and are incapable of in-situ monitoring for the unexpected shocks in wastewater influent (Ikebukuro et al., 1996; Okochi et al., 2004; Neufeld et al., 2006; Eltzov et al., 2009; Woznica et al., 2010). Second, the microorganisms or enzymes coated on the biosensors surfaces have only been tested in less harsh water media (e.g. drinking water, and synthetic pure solutions) and have short life-time (e.g. several hours and days) (Okochi et al., 2004; Curtis et al., 2009; Woznica et al., 2010; Dong et al., 2012) which cannot be used for long-term wastewater quality monitoring. Third, the existing biosensors need external power sources and/or additional probes (e.g. dissolved oxygen, electrical conductivity, and pH) to display the specific parameter changes (Ikebukuro et al., 1996; Konig et al., 1998; Kumlanghan et al., 2008; Oh et al., 2011). This poses difficulty for long-term on-line monitoring in harsh and remote environments (e.g. large Superfund site). Fourth, long response time of biosensors makes it difficult for timely action to minimize the shock impacts. Anaerobic granule biosensors were developed as an early warning system for copper (Cu^{2+}) and phenol in wastewater influent (Jiang et al., 2004). But the response time of 6–20 h makes this biosensor unrealistic for fast response online sensing.

Recently, microbial fuel cell (MFC) (Liu and Logan, 2004), a novel electrobiochemical system capable of converting organic

* Corresponding author.
E-mail address: baikun@engr.uconn.edu (B. Li).

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matters in wastewater to electricity by electrogenic bacteria growing on the electrode surface has been studied as a self-sustainable biosensor for wastewater quality monitoring. Most MFC sensor studies focused on BOD measurement and a good correlation between the power output of MFCs and the BOD concentration (0–300 mg L^{-1}) of wastewater has been established (Kim et al., 2003; Chang et al., 2004, 2005; Moon et al., 2004; Kumlanghan et al., 2007; Lorenzo et al., 2009). The correlations of power output and easily degradable organic matters (e.g. acetate and lactate) by electrogenic bacteria in MFCs were also investigated (Kim et al., 1999; Tront et al., 2008). Until now, most MFC sensor studies have focused on the positive correlation of electrochemical signals of MFCs with organic compounds under a stable operational condition. These MFC sensors have the two-chamber configuration, in which the anode and cathode chambers are separated by a proton exchange membrane (PEM). The anode potential is usually controlled by a potentiostat or the anode current is controlled by a galvanostat and a pH buffer solution to acquire a stable signal baseline (Stein et al., 2010, 2012a, 2012b, 2012c). In fact, MFCs could be an excellent shock sensor for wastewater qualities, since the voltage/power output of MFCs are directly depended on the metabolic activities of the anaerobic electrogenic bacteria (Liu and Logan, 2004a; Liu et al., 2005a; Oh and Logan, 2005; Logan, 2009), and the toxic contaminants in wastewater could instantaneously affect the bacterial activities. Specifically, the sudden presence of toxic contaminants (e.g. heavy metals) or a spike of organic/inorganic substrates concentrations (e.g. nitrate, BOD) are expected to generate a distinct change (sudden jump or drop) in the voltage output of MFCs. Till now, MFC as a shock sensor for wastewater quality has not been systematically explored yet.

MFCs have four unique features as the shock sensors. First, MFCs do not need external enzymes/pure microorganisms loaded, since bacteria in wastewater gradually grow on anode surface and produce the electronic signals. Second, the anaerobic electrogenic bacteria have a fast response to shocks, and the rapid signal change can be used as the early warming for wastewater influent toxic shocks. Third, MFCs generate electricity from wastewater, so that no external power source is needed. This self-sustainable operation makes the long-term online wastewater monitoring possible. Fourth, electrongenic bacteria are expect to have different types of responses to different shocks. For instance, sharp voltage change for acute toxic compounds, while slow voltage change for chronic contaminants. Based on the MFC voltage changing patterns, the toxic shock types could be identified. So far, biosensors (e.g. immobilized microbial membrane (Rastogi et al., 2003) related to wastewater quality monitoring were only studied for one or two parameters or toxins. MFC based biosensors were explored for single toxin that caused the decrease in power output (Stein et al., 2010, 2012a, 2012b, 2012c). A comprehensive investigation of the MFC based sensors for detecting different types of shocks in wastewater still remains elusive.

In this study, a single-chamber batch-mode cube MFC (terms as CMFC) was examined as a sensitive "shock" sensor with a reasonable selectivity for wastewater quality monitoring. By removing the PEM, anode and cathode in CMFCs are exposed to the same wastewater solution, so that the electrogenic bacteria growing on anode surface could rapidly respond to the wastewater quality changes. CMFCs have been studied for biofilms growth on anode (Liu and Logan, 2004; Sharma and Li, 2010), and wastewater treatment (Liu et al., 2004). Four types of shocks were examined by injecting the shock solutions to CMFCs, specifically, chromium (Cr^{6+}) as the acute toxin, iron (Fe^{3+}) as non-toxic metal, nitrate as oxidant substances, and acetate as organic substrate in wastewater. There were four tasks in this study. First, Cr^{6+} (1 and 8 mg L^{-1}) and Fe^{3+} (1, 8, and 48 mg L^{-1}) were examined as the metal shocks in wastewater influent, and the voltage changes of CMFCs were correlated with the shock types. Second, nitrate (1, 8, and 48 mg L^{-1}) and acetate (200 mg L^{-1}) were examined as wastewater quality fluctuation, and the voltage changes were correlated with the non-toxic substance shocks (nitrogen and organic carbon). Third, the control tests were conducted by injecting air and water individually to CMFCs to check whether the voltage of CMFCs could maintain stable at the blank changes in wastewater. Finally, the anode open circuit potential (OCP) was measured after each shock test and correlated with the voltage changes of CMFCs and shock types.

2. Materials and methods

A single chamber CMFC (size: 4 cm × 3 cm × 3 cm with a working volume of 30 mL) was constructed using plexiglass (Fig. 1). The cathode (size: 3 cm × 3 cm) was made of the 4-layer polytetrafluoroethylene (PTFE) treated carbon cloth facing air and the 0.5 mg cm^{-2} Platinum (Pt) loaded side facing water in the CMFC. The Pt was used as the cathodic catalyst for oxygen reduction reaction (ORR) (Cheng et al., 2006). The anode (size: 3 cm × 3 cm) was made of non-PTFE treated carbon cloth and was placed in the opposite side of the cathode in the CMFC. The anode and cathode was connected using a copper wire with an external resistance (R_{ext}: 480 Ω). The voltage output was recorded on the R_{ext} using a Keithly 2700 data logging system.

Fresh wastewater (Chemical oxygen demand (COD): 250–350 mg L^{-1}) taken from the influent at the University of Connecticut Wastewater Treatment Plant contained sufficient microorganism and was used as the inoculum for bioanodes and constantly fed to the CMFC. Four types of shocks to the CMFC were conducted by injecting the targeted chemicals using a syringe into the wastewater solution of the anode chamber. A rubber septum capped on an opening on the top of the CMFC was used as injection point (Fig. 1). Chromium (VI) is a well-known toxic meal to microorganisms, with the limitation of in drinking water 0.1 mg L^{-1} (EPA 816-F-09-0004). The Cr shocks were examined at a low concentration (1 mg L^{-1}) to a high concentration (8 mg L^{-1}). Specifically, 60 μL K_2CrO_4 solution (500 mg L^{-1} Cr^{6+}) was injected to achieve the 1 mg L^{-1} Cr^{6+} shock in the CMFC. The dilution effect can be ignored since the injection volume (60 μL) only counts for 0.2% of the CMFC volume. 40 μL K_2CrO_4 solution (6 g L^{-1} Cr^{6+}) was injected to simulate the 8 mg L^{-1} Cr^{6+} shock in

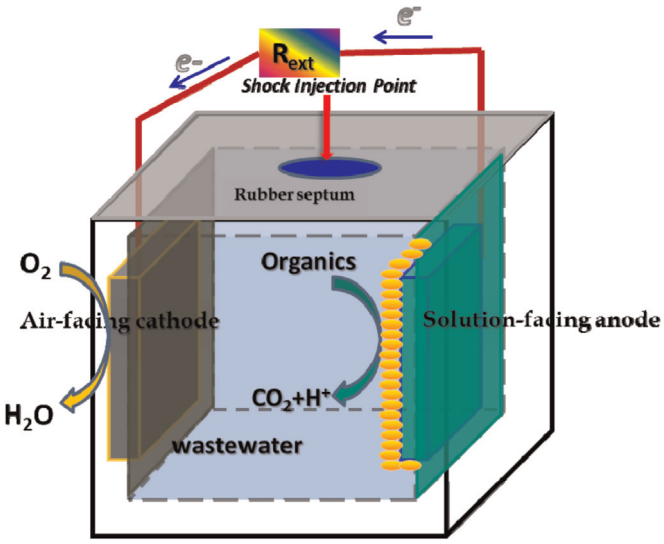

Fig. 1. Schematic depiction of the single chamber CMFC as the shock sensor.

the CMFC. The iron toxicity (Fe^{3+}) to microorganisms is much lower than that of Cr^{6+}, with the limitation of 100 mg L^{-1} in wastewater discharge. Three Fe^{3+} concentrations were examined (1, 8, and 48 mg L^{-1}). Specifically, 60 μL $FeCl_3$ solution (500 mg L^{-1} Fe^{3+}), and 40 μL and 240 μL $FeCl_3$ solution (6 g L^{-1} Fe^{3+}) were injected to simulate 1, 8, and 48 mg L^{-1} Fe^{3+} shocks, respectively.

Nitrate, the product of nitrification, is the electron acceptor for denitrification. Nitrate in the effluent of WWTPs could range up to 30 mg L^{-1}, and has a limitation of 10 mg L^{-1} in drinking water (EPA 816-F-09-0004). Three nitrate concentrations were selected to simulate the nitrate shock in wastewater. Specifically, 60 μL $NaNO_3$ solution (500 mg L^{-1} NO_3^-), 40 μL and 240 μL $NaNO_3$ solution (6 g L^{-1} NO_3^-) were injected to achieve 1, 8, and 48 mg L^{-1} NO_3^- shocks in the CMFC, respectively. The fluctuation of organic contaminants in wastewater influent easily affects the microbial activity in WWTPs. Sodium acetate (NaAc) was selected to simulate organic contaminants in municipal wastewater. The NaAc concentration increase could lead to higher electrogenic bacteria activity and higher voltage output in MFCs (Liu et al., 2005a; Ren et al., 2007; Virdis et al., 2010). The 60 μL NaAc solution (100 g L^{-1}) was injected into the CMFC to simulate 200 mg L^{-1} organic shock. All the shock tests were duplicated.

Throughout the experiments, the anode open circuit potential (OCP) was measured before and after each shock test using a multimeter with anode as the working electrode and an Ag/AgCl reference electrode. The anodes/cathode surfaces were observed using a scanning electron microscope (SEM) before and after the shock tests.

3. Results and discussion

3.1. Voltage responses of CMFC to Cr^{6+} shocks

The voltage responses of the CMFCs to Cr^{6+} shocks were examined at two concentrations (1 mg L^{-1} (Fig. 2a) and 8 mg L^{-1} Cr^{6+} (Fig. 2b)). The first 1 mg L^{-1} Cr^{6+} shock (at 74 min) caused a clear drop in the voltage signal from 0.109 V to 0.091 V, but quickly recovered to 0.098 V and then gradually decreased. The second 1 mg L^{-1} Cr^{6+} shock took place 60 min after the first 1 mg L^{-1} Cr^{6+} injection. A distinct voltage drop (from 0.089 V to 0.081 V) was observed, and the voltage then sharply decreased. After 36 min, the decreasing trend stopped and the voltage began to recover. After 52 min, the voltage reached the plateau, probably because the amount of Cr^{6+} was not enough to deactivate all the electrogenic bacteria in CMFCs.

The control tests with 60 μL air and 60 μL distilled water were individually conducted (Fig. 2a) to determine whether the voltage of CMFCs changed for the blank injections (not the real shocks in wastewater). The results showed that the air injection just caused a light voltage drop (from 0.110 V to 0.106 V) (at 42 min, Fig. 2a), compared with the clear voltage drop from Cr^{6+} injection. Because the electrogenic bacteria in MFCs are anaerobic bacteria (Mohan et al., 2008; Logan, 2009), the air injection introduced oxygen and partially inhibited the electrogenic bacterial activity, and led to the slight voltage drop. The distilled water injection caused no distinct voltage drop (at 58 min, Fig. 2a). The good stability of CMFCs at these two blank injections demonstrated the feasibility for shock sensors, since CMFCs had clear sharp voltage drop at the toxic shock (e.g. 1 mg L^{-1} Cr) while kept the stable voltage at the air and water injections. Because the Cr concentration limitation is 0.1 mg L^{-1} in drinking water (EPA 816-F-09-0004), the good sensitivity of CMFCs at 1 mg L^{-1} Cr shock revealed the feasibility for monitoring shocks in wastewater quality.

Anode OCP, an indication of the activity of anodic electrogenic bacteria, was recorded after each shock test. Typical anode OCP of the CMFCs treating wastewater (without the chemical shock injection) was ranged between −350 and −398 mV (Table 1). The OCP value higher than this negative value indicated a lower bacterial electrogenic activity (Santoro et al., 2012, 2013). The anode OCPs after two successive 1 mg L^{-1} Cr^{6+} injections was −298 mV, which was well correlated with the toxicity of Cr and the voltage drop of CMFCs (Table 1).

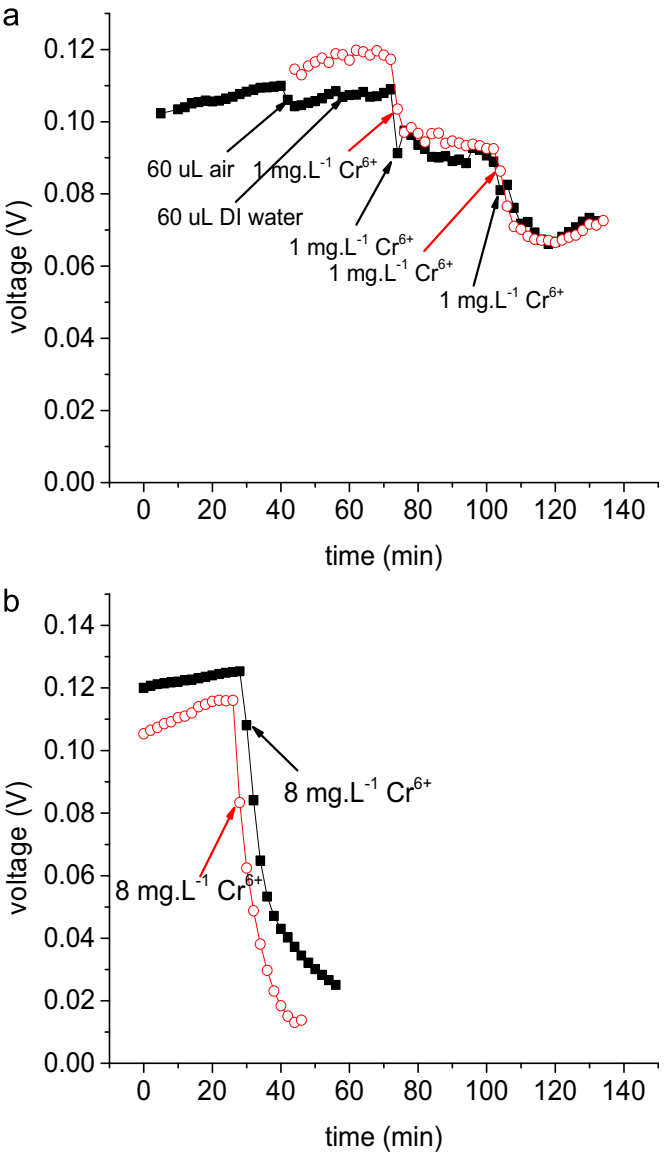

Fig. 2. Voltage responses of CMFC to blank changes (air and distilled water), 1 mg L^{-1} Cr^{6+} shock (a) and 8 mg L^{-1} Cr^{6+} shock (b). (Solid dots and hollow dots represent the duplicate tests.)

Table 1
The anode OCP values before and after the shocks.

Shock type	No shock	[a]2 mg L^{-1} Cr^{6+}	8 mg L^{-1} Cr^{6+}	[a]57 mg L^{-1} Fe^{3+}	[a]57 mg L^{-1} Nitrate	200 mg L^{-1} NaAc
Anode OCP (−mV)	350–398	298	19	266	355	399

[a] The chemical concentrations were the accumulated value at the time point the OCPs were measured.

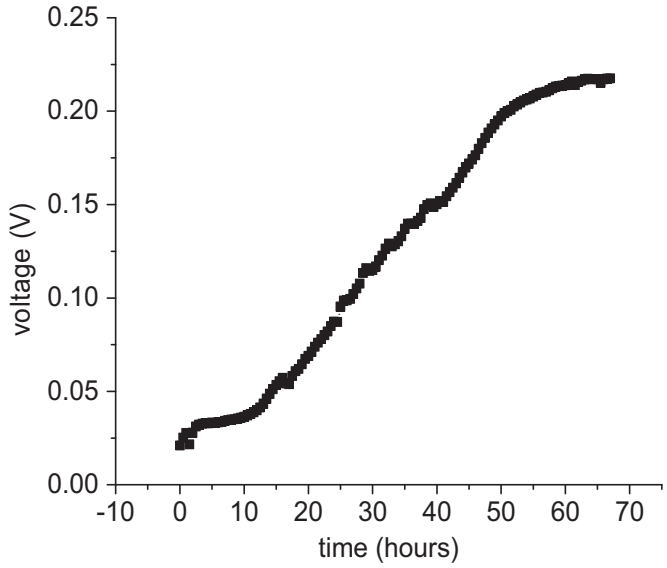

Fig. 3. Anode recovery in the CMFC after the 8 mg L^{-1} Cr^{6+} shock test.

Higher concentration Cr^{6+} shock (8 mg L^{-1}) was conducted independently from the 1 mg L^{-1} Cr^{6+} shock test (solid dots, Fig. 2b). The *x*-axis scale (time duration) was kept the same as Fig. 2a to compare the shock extents of 8 mg L^{-1} and 1 mg L^{-1} Cr^{6+}. A steep voltage drop was observed after the shock taking place at 30 min. Within the next 10 min, the voltage substantially decreased from 0.125 V to 0.053 V, and then steadily dropped to 0.02 V after 20 min. The 8 mg L^{-1} Cr shock caused faster and more dramatic drop in voltage signals than the 1 mg L^{-1} Cr shock. The anode OCP (–19 mV, Table 1) clearly indicated that most of the electrogenic bacteria were inactivated by this high concentration Cr^{6+}. The duplicate tests (hollow dots, Fig. 2b) had the similar voltage change trend, indicating that the CMFC response to 8 mg L^{-1} Cr^{6+} shock was repeatable.

The reusability of biosensors is critical for shock tests. If all the bacteria in MFCs are inactivated by toxic shocks without recovery, the biosensors have to be replaced after the toxic shock, which poses a potential problem for real-world applications. In this study, although most of the bacteria were deactivated after the 8 mg L^{-1} Cr^{6+} shock, the anode bacterial activity was able to be recovered with feeding wastewater containing 1 g L^{-1} $NaNO_3^-$, indicated by a steady voltage increase after 65 h (Fig. 3). This result proved that the CMFC shock sensor can be recovered after a toxin shock (inactivating most anodic bacteria) albeit a relatively long revival time required.

3.2. Voltage responses of CMFC to Fe^{3+} shocks

The voltage responses of the CMFC to the Fe^{3+} shocks (in the form of $FeCl_3$) were much milder than to Cr^{6+} shocks (Fig. 4a). The 1 mg L^{-1} Fe^{3+} shock only caused a slight voltage drop from 0.121 V to 0.118 V, and then the voltage recovered within 30 min. The 8 mg L^{-1} Fe^{3+} shock at 60 min caused the voltage to gradually drop within next 10 min (Inset 1, Fig. 4a). The 48 mg L^{-1} Fe^{3+} shock took place at 90 min had a similar trend to the 8 mg L^{-1} Fe^{3+} shock, and the voltage gradually dropped to 0.067 V at 150 min (Inset 2, Fig. 4a). The anode OCP value measured at the end of the 48 mg L^{-1} Fe^{3+} was −266 mV (Table 1), which was higher than that of wastewater along (−350 and −398 mV) but much lower than that of Cr^{6+} shocks (−19 mV). This implied that high Fe^{3+} concentration could deactivate some of the electrogenic bacteria, but the majority of electrogenic bacteria survived and the voltage only gradually dropped, unlike the sharp drops at Cr^{6+} shock (Fig. 2b). Moreover, electrogenic bacteria play an important role in Fe^{3+} reduction (e.g. anaerobic sediment) (Lovley and Phillips, 1986; Lovley, 1991). The electrons generated on anode

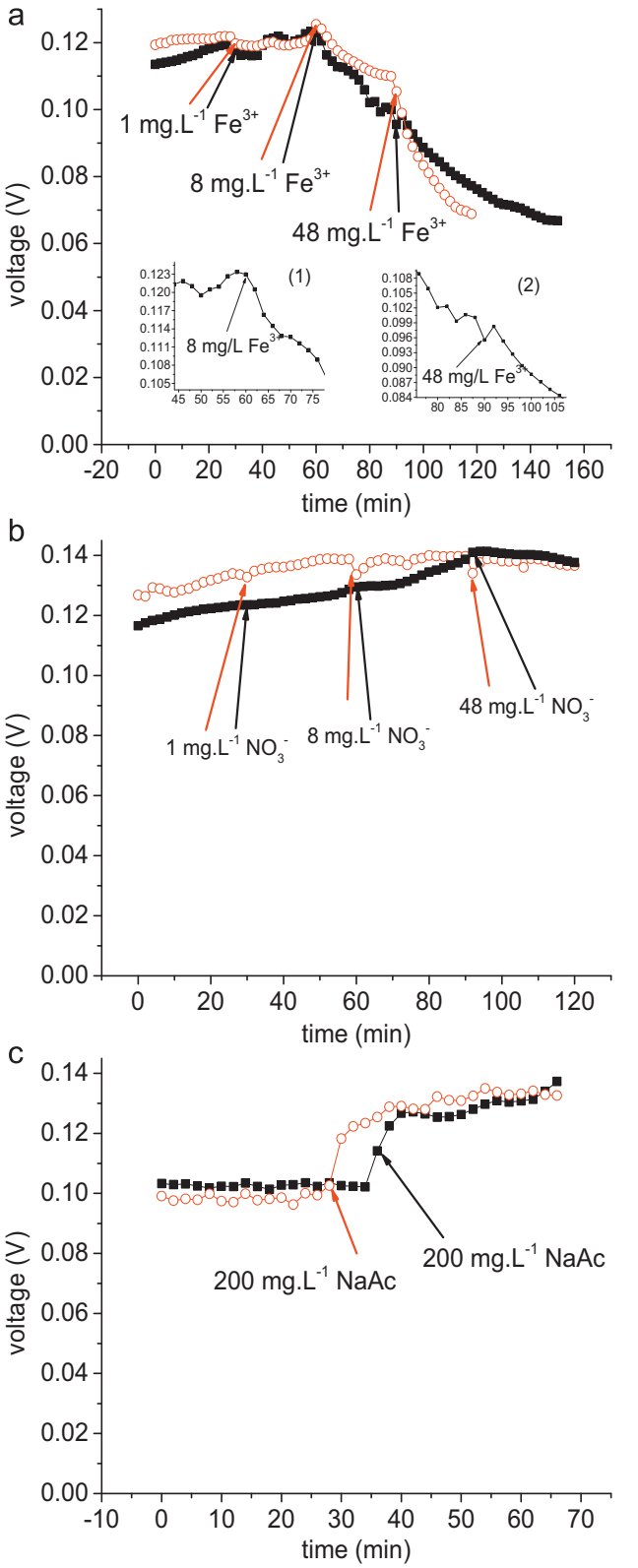

Fig. 4. Voltage responses of CMFCs to non-toxin shocks. (a) 1, 8 and 48 mg L^{-1} Fe^{3+} shocks (Inset 1: Zoom in of the 8 mg L^{-1} Fe^{3+} shock point. Insert 2: Zoom in of the 48 mg L^{-1} Fe^{3+} shock point). (b) 1, 8, and 48 mg L^{-1} NO_3^- shocks and (c) 200 mg L^{-1} NaAc shocks. (Solid dots and hollow dots represent the duplicate tests.)

electrodes could be directly consumed by Fe^{3+} reduction in the anode solution, rather than going to cathode electrodes for oxygen reduction reaction (ORR). At the low Fe^{3+} concentration (e.g. 1 mg L^{-1} in this study), the Fe reduction reaction did not consume significant amounts of electrons, so that the voltage just fluctuated after the injection. With the Fe^{3+} concentration kept increasing to 8 mg L^{-1} and 48 mg L^{-1}, the Fe reduction reaction started consuming high amounts of electrons and led to the steady drop of voltage. The duplicate test (hollow dots, Fig. 4a) followed the same voltage trend for all Fe^{3+} shocks.

The voltage trends at Cr^{6+} and Fe^{3+} shocks were distinctly different. For Cr^{6+} shock, even low concentration (1 mg L^{-1}) could lead to the instantaneously sharp voltage drop. For Fe^{3+} shock, low concentration (1 mg L^{-1}) only caused the slight voltage fluctuation, and high concentration (8 and 48 mg L^{-1}) led to the steady voltage drop. This voltage declining pattern could distinguish the shocks of acute toxic contaminants (e.g. Cr) or the shocks of normal contaminants (e.g. Fe as the electron acceptor) in wastewater.

3.3. Voltage responses of CMFC to NO_3^- shocks

Similar to Fe^{3+}, nitrate (NO_3^-) is also a favorable electron acceptor due to the high oxidative status of N in NO_3^-. It was examined as a shock to the CMFC at the same concentrations as Fe^{3+} (1, 8, 48 mg L^{-1} NO_3^-). However, quite different from the Fe^{3+} shock results, the voltage had no distinct change at the three shocks at 30 min (1 mg L^{-1} NO_3^-), 60 min (8 mg L^{-1} NO_3^-), and 90 min (48 mg L^{-1} NO_3^-), respectively. The voltage displayed a smooth pattern within the time range studied (0–120 min) and increased from 0.117 V at the initial point to 0.138 V at the end point (solid dots, Fig. 4b). These phenomena indicated that 1, 8, and 48 mg L^{-1} NO_3^- were unable to give a distinct shock to the CMFC voltage output. The trend of the voltage generation was changed from slow increase to slow decrease at the 48 mg L^{-1} NO_3^- shock. Denitrification has been studied in the cathode chamber of the 2-chamber MFCs (Clauwaert et al., 2007; Virdis et al., 2010) and the NO_3^- (as electron acceptor) was removed. The slow voltage drop after the 48 mg L^{-1} NO_3^- injection could imply that the electrons were directly consumed by the accumulated NO_3^- (57 mg L^{-1}, Table 1) in the anode solution, instead of transporting to the cathode through the external circuit. In addition, the anode OCP of −355 mV after the NO_3^- shock tests implied the bacterial activity on anodes was barely affected by NO_3^-. Nitrate did not have toxic effects on bacteria, which was quite different from the Fe^{3+} shocks (anode OCP: 266 mV). The duplicate shock tests (hollow dots, Fig. 4b) showed the same trend, with negligible voltage change at 1 mg L^{-1} NO_3^- shock and slight fluctuation at 8 and 48 mg L^{-1} NO_3^- shocks.

3.4. Voltage responses of CMFC to sodium acetate shocks

Acetate was examined as the shocks of organic contaminants in wastewater that acts as the fuel for MFCs. Acetate shock tests were conducted at the fuel depleting stage of the CMFC. The sodium acetate (NaAc) solution (concentration: 200 mg L^{-1}) was injected to the CMFC at 36 min (solid dots, Fig. 4c). The voltage sharply jumped from 0.102 V to 0.114 V within 2 min and to 0.122 V within 4 min, and kept increasing for the rest 26 min. The anode OCP (−399 mV) exhibited a jump from wastewater alone (−355 mV) (Table 1). This increase in the absolute value of OCP indicated that the electrogenic bacteria were enriched with the presence of 200 mg L^{-1} NaAc. Higher organic substrates led to higher voltage output of MFCs (Jiang et al., 2010; Sharma et al., 2011; Santoro et al., 2011). The ionic strength increase caused by the NaAc shock could also cause the voltage increase (the instant increment of ionic strength due to the addition of 200 mg L^{-1} sodium acetate was 0.0048 mol L^{-1}) (Liu et al., 2005b). Nevertheless, $NaNO_3$ in the NO_3^- shock test also increased the ionic strength (the instant increment of ionic strength due to the addition of 48 mg L^{-1} NO_3^- was 0.0015 mol L^{-1}), but a slow voltage decrease was observed after 48 mg L^{-1} NO_3^- shock (Fig. 4b), which confirmed that the voltage increase in the NaAc shock test was mainly caused by the higher electrogenic bacterial activity at higher organic substrate concentration. The NaAc shock test demonstrated that the CMFC was able to detect the high organic shocks in wastewater. The duplicate tests (hollow dots in Fig. 4c) followed the same trend, indicating that the NaAc shock signal was repeatable.

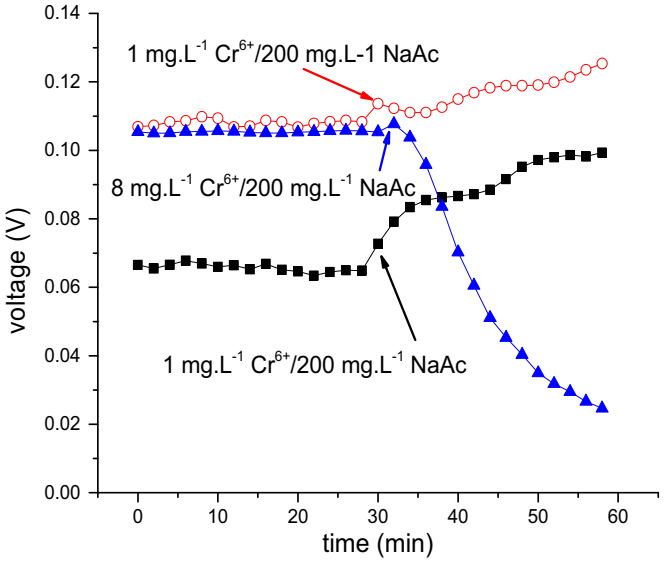

Fig. 5. Voltage responses of CMFCs to combined shocks. Square dots: 1 mg L^{-1} Cr^{6+}/200 mg L^{-1} NaAc shocks (solid square dots and hollow round dots represent the duplicate tests). Triangle dots: 8 mg L^{-1} Cr^{6+}/200 mg L^{-1} NaAc shocks.

3.5. Voltage responses of CMFC to Cr^{6+}/sodium acetate combined shocks

In addition to individual shocks, the combined shocks of toxins (e.g. Cr^{6+}) and organic substances (e.g. NaAc) could simultaneously happen in wastewater influent. The coupling of fuels with toxins might weaken the CMFC responses to toxins, or even mask the response and cause false signal. In this study, this situation was simulated by putting the Cr^{6+} (toxin)/NaAc (fuel) combined shocks to CMFCs. Specifically, the 200 mg L^{-1} NaAc/1 mg L^{-1} Cr^{6+} mixture was injected at 30 min at the fuel depleting stage of the CMFC. The voltage experienced a sharp increase (solid square dots, Fig. 5), which was similar to the single 200 mg L^{-1} NaAc shock test (Fig. 4c), but was different from the single 1 mg L^{-1} Cr^{6+} shock (Fig. 2a). This clearly indicated that the positive effect of 200 mg L^{-1} NaAc surpassed the toxic effect of 1 mg L^{-1} Cr^{6+} on electrogenic bacterial activity. The 200 mg L^{-1} NaAc/1 mg L^{-1} Cr^{6+} combined shock was duplicated (hollow round dots, Fig. 5) at the fuel abundant stage of the CMFC. Interestingly, a small voltage increase was observed at 30 min and followed by a slow voltage increase, which was distinctly different from single 1 mg L^{-1} Cr^{6+} shock (Fig. 2a) and single 200 mg L^{-1} NaAc shock (Fig. 4c). This might indicate that the availability of fuels (organic substances) to the anodic electrogenic bacteria affected the signals of the combined shocks.

A 200 mg L^{-1} NaAc/8 mg L^{-1} Cr^{6+} combined shock was also injected to simulate the shock of higher amount toxin coupling with fuel (solid triangle dots, Fig. 5). The voltage experienced a

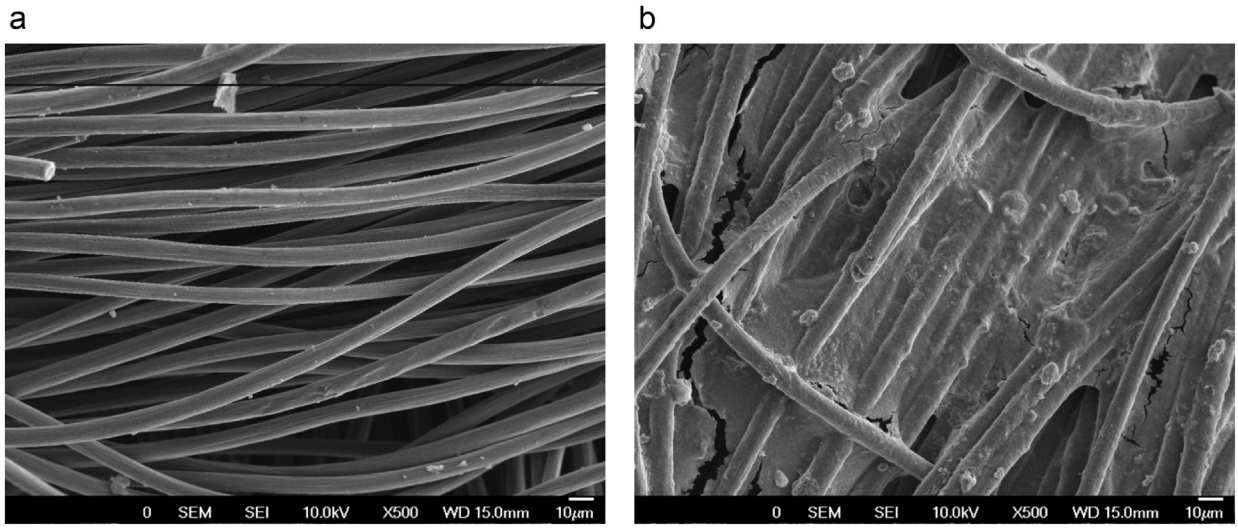

Fig. 6. The SEM images of anodes in the CMFC. (a) Clean carbon cloth; (b) biofilms growing on carbon cloth.

small increase at the shock point but then quickly dropped to 0.02 V in 30 min, which was similar to single 8 mg L^{-1} Cr^{6+} shock tests (Fig. 2b). The small increase in the first 2 min could be caused by a sudden increase in NaAc, and this effect also slowed down the decreasing trend of the voltage at the 8 mg L^{-1} Cr^{6+}. The toxic effect of 8 mg L^{-1} Cr^{6+} still overwhelmed the positive effect of the 200 mg L^{-1} NaAc in a short time period and inactivated most of the electrogenic bacteria afterwards in 30 min.

3.6. Biofilm growth on bioanodes

The SEM images of the clean carbon cloth anode (Fig. 6a) and the carbon cloth anode after 5-day shock tests (Fig. 6 b) clearly showed that anodic biofilms were fully grown on anode surfaces after operated in wastewater for several days. Unlike 2-chamber MFCs that separate anodic electrogenic bacteria from cathodic toxins with proton exchange membrane (PEM), biofilms growing on anodes of the single chamber CMFCs were directly exposed to toxins in wastewater. Thus, the anodic biofilms acted as the supportive and protective structure for electrogenic bacteria, and make it possible for anodic bacteria to revive from toxin shocks in wastewater.

3.7. Selectivity of shock sensors and significance for wastewater real-time monitoring

The main function of the "selective shock" CMFC sensor developed in this study is to detect the "presence/absence" of toxic matters in wastewater influent, and differentiate toxic shocks from non-toxic shocks. It is not designed to measure the toxic/nontoxic concentrations in wastewater (e.g. low concentration, Cr^{6+} 0.1–0.5 mg L^{-1}). In this study, the voltage significantly dropped at 1 and 8 mg L^{-1} toxic Cr^{6+}, while did not noticeably change at 1, 8 and 48 mg L^{-1} non-toxic NO_3^-. Because Fe^{3+} is less toxic compared with Cr^{6+}, the 1 mg L^{-1} Fe^{3+} did not change the voltage. The 8 mg L^{-1} Fe^{3+} started to exhibit toxicity (indicated by the change of anode OCP) and both 8 and 48 mg L^{-1} Fe^{3+} caused the distinct voltage drop. The voltage response showed that even the 48 mg L^{-1} Fe^{3+} was less toxic than 8 mg L^{-1} Cr^{6+} and caused a much less voltage drop and a much lower anode OCP. In addition, NaAc that favors the growth of the electrogenic bacteria on the anode gave a distinct increase in voltage. It should be noted that even though the "selective shock" CMFC sensor is not aimed at measuring toxins at low concentrations, the results showed that 1 mg L^{-1} Cr^{6+} led to less voltage drop than 8 mg L^{-1} Cr^{6+}; thus it could be logically assumed that low concentrations of toxins would give less voltage drop.

The CMFC shock sensor developed was able to effectively differentiate four types of shocks in wastewater influent within 5 min. For the first time, the CMFC was found to be capable of detecting Cr^{6+} as low as 1 mg L^{-1}, which indicates a high sensitivity for toxic contaminants in wastewater. More importantly, the voltage changing patterns vary with the type of shocks, which can be used to distinguish the type of shocks (acute toxic shock or non-toxic spike). This early warning system is critical to determine the wastewater influent shock types, and provide timely precaution and solution. This study demonstrates the distinct advantages of CMFC shock sensors. No external power supply was needed for MFC shock sensors, which provides high flexibility and stability for long-term monitoring. Compared to other MFC sensors to measure contaminant concentrations (e.g. BOD and nitrogen), this CMFC shock sensor does not require a fixed anode potential/current controlled by a potentiostat or a galvanostat, which solves the complexity of sensors. No supplemental probes (e.g. pH, Oxygen probe) are needed, since the sensing signal (voltage output) is directly generated from wastewater treatment in the CMFC. This batch-mode CMFC study reveals that MFC shock sensors hold a great potential to be applied as a real time early warning sensor for wastewater quality monitoring. Further continuous flow CMFCs will be conducted to examine the sensitivity for wastewater influent shocks.

4. Conclusions

A single chamber CMFC was examined as a real time sensor to detect four types of chemical shocks in wastewater influent. The batch-mode tests showed that the CMFC effectively differentiated Cr^{6+}, Fe^{3+}, NO_3^- and NaAc shocks. Specifically, Cr^{6+} of low concentration range gave distinct voltage drop, compared with relatively higher concentrations of Fe^{3+}. As non-toxic compound, NO^{3-} was unable to excite clear voltage change. NaAc resulted in a sharp increase in the voltage signal, since it is a favorable organic substrate to anaerobic electrogenic bacteria in MFC. The Cr^{6+} (8 mg L^{-1})/NaAc (200 mg L^{-1}) mixture shock caused a sharp voltage drop within 30 min.

The anode OCP change was clearly correlated with the voltage changes of CMFCs, indicating the voltage change was mainly

caused by the anodic electrogenic bacteria activity. The CMFC was able to recover its sensing function after 65 h of start-up (anodic bacteria acclimation) time with feeding wastewater and 1 g L^{-1} NaAc. The CMFC inoculated with wastewater is a promising prototype for real time, self-powered toxicity shock sensor for wastewater quality monitoring.

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