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# Sequential flowing membrane-less microbial fuel cell using bioanode and biocathode as sensing elements for toxicity monitoring

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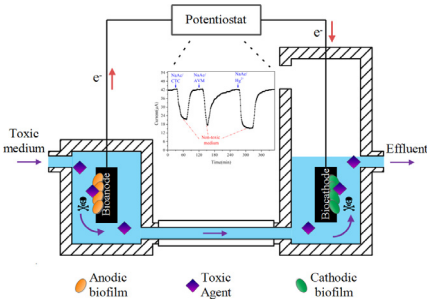
## GRAPHICAL ABSTRACT

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## ABSTRACT

Traditional microbial fuel cell based biosensor (MFC-Biosensor) utilizes bioanode as sensing element and delivers high sensitivity for single toxic shock but it fails to alert the combined shock of organic matter (OM)/toxic agent (TA). To address this limitation, this study developed a sequential flowing membrane-less MFC based biosensor (SMFC-Biosensor) using both bioanode and biocathode for toxicity monitoring. Results demonstrated the shocks of 1.5 mg/L $Hg^{2+}$, 1.0 mg/L avermectin and 1.0 mg/L chlortetracycline hydrochloride to SMFC-Biosensor led to inhibition ratios of 36%, 15% and 9%, which were over twice higher than those of bioanode-based and biocathode-based MFC-Biosensors. The viabilities of anodic and cathodic biofilms were both inhibited by the toxic shock. Besides, the excessive organic matters caused a decay in the SMFC-Biosensor current and consequently the OM/TA combined shock could be successfully monitored. This study for the first time testified the feasibility of simultaneously using bioanode and biocathode as sensing elements for toxicity monitoring.

## 1. Introduction

With the advancement of industrialization, the extensive use of chemical products and human activities have caused serious water contamination. Toxic pollutants such as heavy metals could directly destroy the ecological environment of water body. Hence, the development of water quality monitoring method is critical to guarantee the water supply safety. Various types of biosensors have been employed to monitor the biotoxicity of water sample. During the past decade, there is an increasing interest in the research of microbial fuel cell based biosensors (MFC-Biosensors). With a simple construction and no additional transducer, MFC-Biosensors are applicable for the in-situ

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and on-line monitoring of water quality (Prevoteau and Rabaey, 2017).

MFCs use electricigens as biocatalysts to convert chemical energy in organic matters (OMs) to electricity (Logan, 2009). The electrode biofilms work as the sensing elements of MFC-Biosensors. When toxic agents (TAs) appear in the influent, the electrochemical activity of biofilm would be inhibited and consequently the MFC output signal decays. Therefore, the occurrence of toxic shock can be real-time monitored by tracking the changes of voltage (or current). The majority of current researches focused on using bioanode as sensing element. By the optimization of parameters such as configuration (Di Lorenzo et al., 2014), electrode material (Xu et al., 2016) and control mode (Jiang et al., 2015), the sensor sensitivity towards single toxic shock was greatly enhanced. However, in real world applications the presence of TAs might simultaneously occur with the overload of OMs (noted as the OM/TA combined shock). Within a certain concentration range, the increased OMs would improve the electricity generation ability of anodic electricigens and thus covered the voltage drop induced by the inhibitory effect of TAs on biofilm activities, resulting in a warning failure of the combined shock by the bioanode-based MFC-Biosensor (Liu et al., 2014). A very recent study proposed to eliminate the interference of OM by adding acetate or glucose but this strategy would largely deteriorate the sensor sensitivity, which was unsuitable for practical applications (Tan et al., 2018). Towards this problem, Jiang et al. (2017) first reported using biocathode as sensing element. Due to the adverse impact of OM on cathodic reaction, the biocathode-based MFC-Biosensor could alert the combined shock of NaAc (0.01–0.82 g/L) and formaldehyde (0.0005%). The availability to monitor the OM/TA combined shock and the enhanced sensitivity made this novel sensing element very promising for practical applications.

Sequential flowing membrane-less MFC (SMFC) with biocathode has been employed to remove nitrogen and organic carbon in wastewater treatments (Xie et al., 2014; Kim et al., 2016). For SMFC, the metabolism of anodic and cathodic electricigens both contributes to the electricity generation process. Once a toxic shock occurs, because the anodic effluent is directly introduced into the cathodic chamber, the anodic and cathodic biofilms would both contact with TAs and consequently the biofilm activities would be inhibited, resulting in an attenuated output signal. Based on this principle, the bioanode and biocathode of SMFC could simultaneously work as sensing elements to monitor toxicity and the sensor sensitivity might be improved than single sensing element. A previous study reported that the SMFC didn't suffer from the unceasingly increasing cathode pH problem (Du et al., 2011). Meanwhile, unlike platinum or other metals the cathodic electricigens as renewable biocatalysts were not subject to sulphide poisoning (Freguia et al., 2008). These advantages make SMFC suitable for long-term stable operation, which is indispensable to construct an MFC-biosensor for continuous online monitoring. Moreover, because of the inhibitory impact of excessive OMs on cathodic reaction (Freguia et al., 2008), SMFC might be able to avoid the false alert towards the OM/TA combined shock.

To testify the feasibility of simultaneously using bioanode and biocathode as sensing elements, a small scale SMFC-Biosensor with aerobic biocathode was constructed in this study and the sensor responses to the single shocks of chlortetracycline hydrochloride (CTC), avermectin (AVM) and $Hg^{2+}$ were tested respectively. Then the SMFC-Biosensor was challenged with toxic medium containing increased NaAc and the three TAs to investigate the sensor performance for the OM/TA combined shock. Besides, the effect of toxic shock on biofilm viability was analyzed and the working principle of SMFC-Biosensor was discussed.

## 2. Materials and methods

### 2.1. MFC-Biosensor construction and start up

Two SMFC-Biosensors were developed in this study (Fig. 1). Both

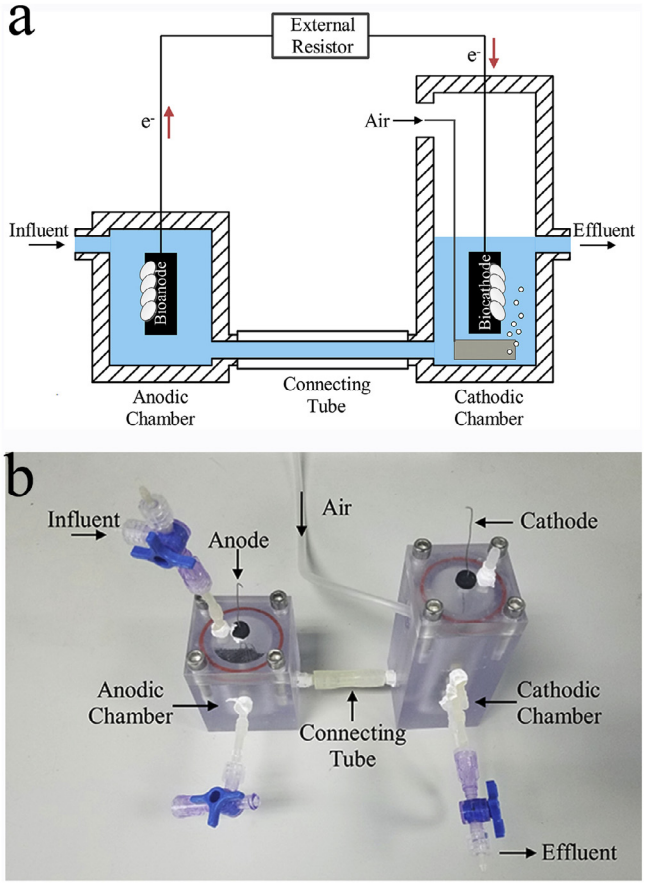


**Fig. 1.** (a) Schematic diagram and (b) prototype of SMFC-Biosensor.

anodic and cathodic chambers had a cylindrical interior with a diameter of 3 cm. The effective height of interior was 4 cm, resulting in a working volume of 28 mL. The two chambers were connected through a silicone tube, which had a diameter of 5 mm and a length of 5 cm. Two 3.0 cm × 2.5 cm pieces of carbon cloth (HCP330, Hesen Electric Corporation, China) were individually used as the anode and cathode. Before installation into SMFC, the two pieces of carbon cloth had separately undergone the enrichment of biofilm in the anodic and cathodic chambers of an MFC which had been stably operated for over two months. The anode and cathode were connected through a resistor of 680 Ω (Yi et al., 2019). Four conventional dual-chamber MFCs were constructed as control, two of which were chemical-cathode MFCs (CMFCs). The other two were biocathode MFCs (BMFCs).

Each liter of medium contained 0.082 g NaAc, 5.85 g NaCl, 0.13 g KCl, 0.31 g $NH_4Cl$, 3.04 g $NaH_2PO_4{\cdot}2H_2O$, 10.92 g $Na_2HPO_4{\cdot}12H_2O$, 12.50 mL trace minerals solution and 5 mL vitamin solution. The catholyte was modified from the medium without NaAc. All the MFCs were operated under continuous flow mode. Fresh medium was pumped into the anodic chambers of SMFC and CMFC via a peristaltic pump (BT100-1L, LongerPump, China) at a flow rate of 2.0 mL/min. Similarly, the catholyte was pumped into the cathodic chamber of BMFC at the same flow rate. Air was continuously sparged into the cathodic chambers of all the MFCs to support oxygen reduction reaction. All the MFCs were incubated at a temperature of 25.0 ± 0.5 °C and the output voltages were recorded by a data acquisition system (USB-1608FS, Measurement Computing Corporation, USA) with a sampling interval of 1 s.

### 2.2. Toxic shock tests

Toxic shock tests were conducted while all the MFCs operated

stably. The test concentrations of CTC, AVM and $Hg^{2+}$ were 1.0 mg/L, 1.0 mg/L and 1.5 mg/L, respectively. For the OM/TA combined shock test, the concentration of NaAc increased from 0.082 g/L to 0.164 g/L. In addition, the toxic shock tests were performed under the constant potential mode to enhance sensor sensitivity (Jiang et al., 2015). MFC was connected to a potentiostat (CHI 1030C, Chenhua Corporation, China) in two-electrode mode. The applied potential was equal to the stable voltage across the resistor. Toxic medium was pumped into the anodic chamber of SMFC at a flow rate of 2.0 mL/min and the toxic shock lasted for 30 min. Then the influent was switched to fresh medium and the next test was conducted after the complete recovery of SMFC current. Each type of toxic shock was tested in duplicates. The bioanode of CMFC and biocathode of BMFC were also challenged with the same toxic shocks to compare the sensor sensitivity. Besides, to analyze the responses of SMFC to OM/TA combined shocks, the SMFC was fed with medium containing different concentrations of NaAc and the effect of excessive OMs was studied. During all the tests, the inhibition ratio (IR) of current was calculated according to Kim et al. (2007) to assess the sensitivity to toxic shock.

### 2.3. Analysis of biofilm viability

After the toxic shock tests described in Section 2.2 were all conducted, a second AVM shock was performed and the anodic biofilms of SMFC and CMFC as well as the cathodic biofilms of SMFC and BMFC were sampled before and after the shock to investigate the effect of toxic shock on electrode biofilm viability. The size of biofilm sample was 0.5 cm × 0.5 cm. All the samples were stained with the LIVE/DEAD® BacLight™ Bacterial Viability Kit (7012, Invitrogen, USA) according to the protocol. Confocal laser scanning microscope (CLSM) images of the biofilm samples were acquired and the ratio of live bacteria to total biomass (noted as biofilm viability) was calculated with reference to a previous study (Li et al., 2016).

## 3. Results and discussion

### 3.1. SMFC-Biosensor responses to single toxic shocks

After the start up process, all the MFCs generated stable currents with fluctuation less than 2%. Then the SMFC-Biosensor was challenged with the three types of single toxic shocks and the sensor responses were presented in Fig. 2a. As the influent was switched to the toxic medium containing 1.0 mg/L CTC, an apparent drop in output current was observed. The current rapidly decreased from 42.59 μA to 40.29 μA within 10 min and afterwards it still showed a slow downward trend, resulting in an IR of 9%. In contrast, the CTC shock only caused a slight decline in the CMFC current with an IR of 3% while the BMFC current barely changed (Fig. 2b). This phenomenon suggested a higher sensitivity of SMFC-Biosensor to the CTC shock. Besides, the SMFC current quickly recovered when fresh medium was again pumped into the anodic chamber. A complete recovery was achieved within 30 min, indicating the damage of CTC shock to biofilm was reversible.

When the output currents of all the MFCs stabilized again, the responses to the shocks of AVM and $Hg^{2+}$ were further investigated. Compared with the response to CTC, sharper decreases in SMFC and CMFC currents were observed under the two shocks (Fig. 2a). The IR values of SMFC to 1.0 mg/L AVM and 1.5 mg/L $Hg^{2+}$ were 15% and 36%, respectively, which were over twice higher than those of CMFC (Fig. 2b). Interestingly, it was specially noticed that the BMFC current unexpectedly increased by 6% under the shock of $Hg^{2+}$ (Fig. 2b). This phenomenon indicated the presence of $Hg^{2+}$ in the cathodic chamber probably triggered a new electrochemical reaction, which affected the electron transfer process. Two types of reactions might simultaneously occur on the cathode: (i) the catalytic activity of cathodic electricigens to reduce oxygen was inhibited, deteriorating the ability to utilize electrons; (ii) due to the relatively high redox potential, $Hg^{2+}$ acted as

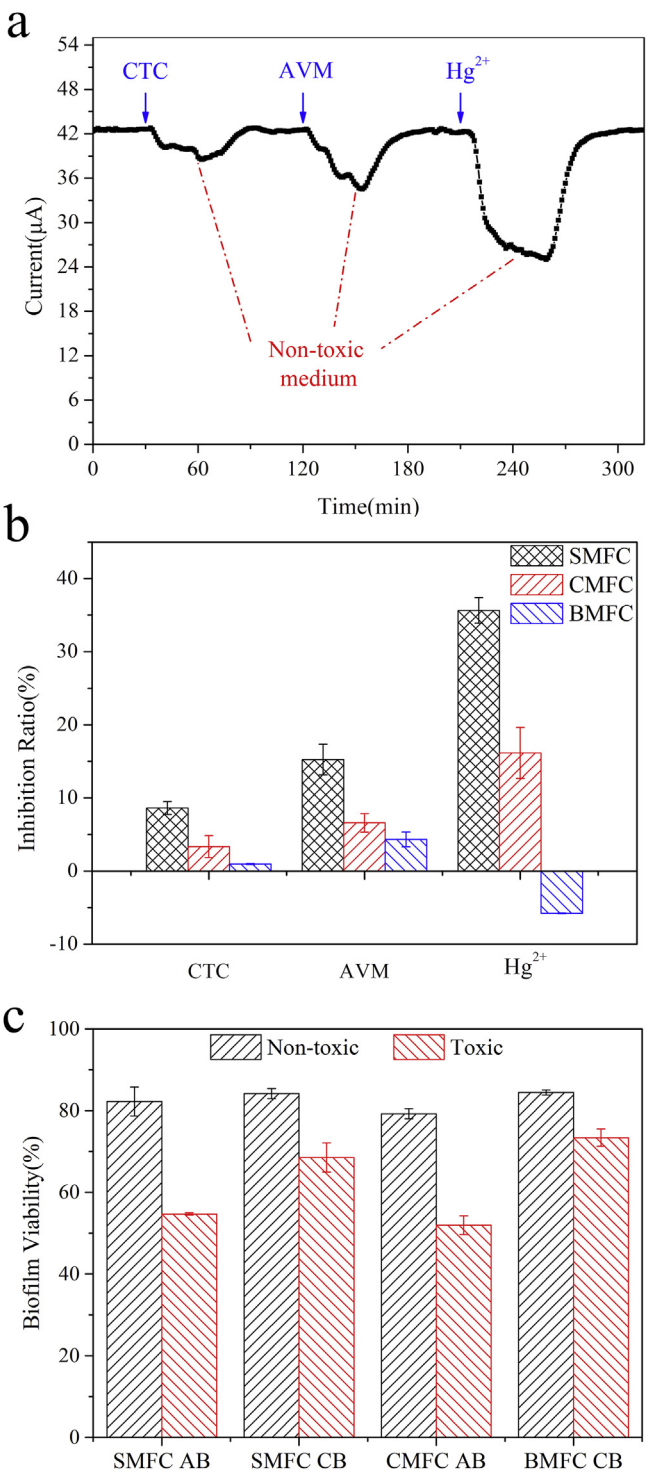


**Fig. 2.** Effect of single toxic shock on SMFC-Biosensor current and biofilm viability. (a) SMFC-Biosensor responses and (b) IR values. (c) Biofilm viabilities under non-toxic and toxic (AVM shock) conditions. AB: anodic biofilm, CB: cathodic biofilm.

an additional electron acceptor (Wang et al., 2011), enhancing the ability to receive electrons. The rates of these two reactions together determined the output current of BMFC.

CLSM images clearly showed the effect of toxic shock on electrode biofilm viability. Large numbers of live bacteria wrapped along the carbon fibers under non-toxic condition, while more dead biomass was observed after exposed to AVM for 30 min. It was found that the anodic biofilm viability of SMFC decreased from 82.25% to 54.69%, which was

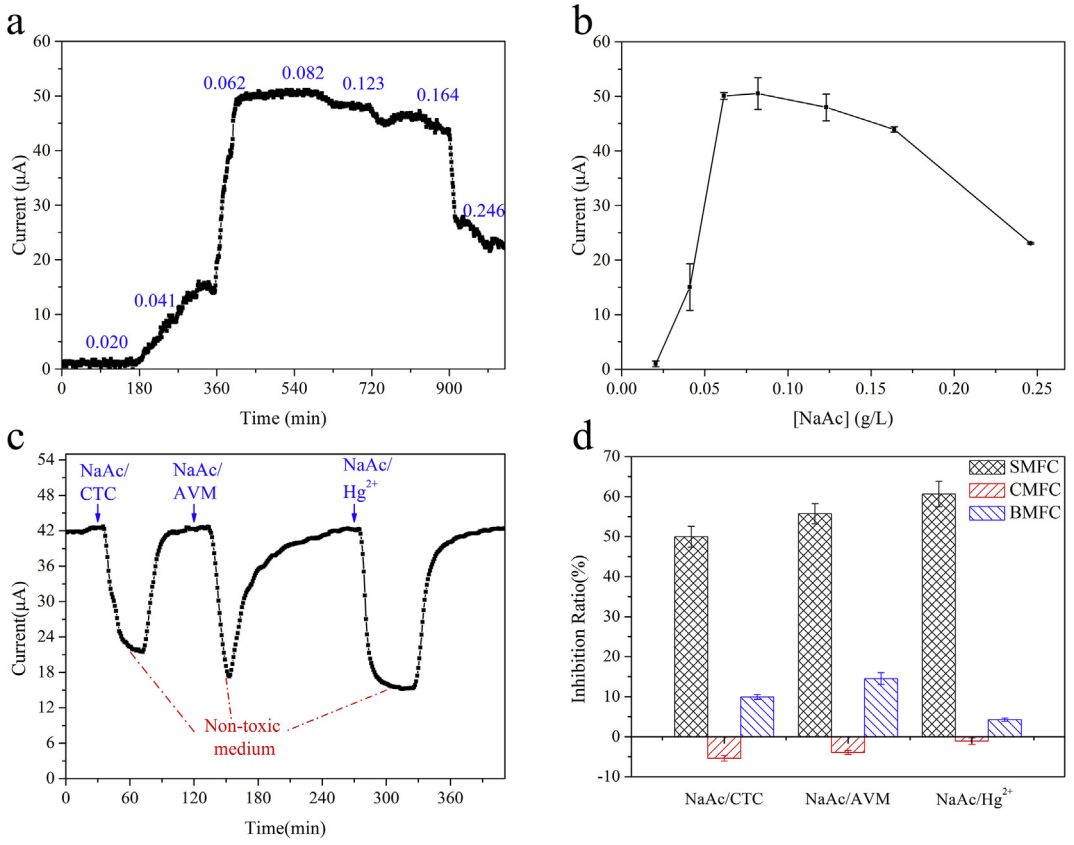


**Fig. 3.** Effects of OM and OM/TA combined shocks on SMFC signal. (a) Current responses to various concentrations of NaAc and (b) stable currents under each concentration. (c) Current responses to OM/TA combined shocks and (d) IR values.

similar to that of CMFC (Fig. 2c). As for the cathodic biofilm, it dropped by 15.65% and 11.08% for SMFC and BMFC, respectively. However, the IR of SMFC to AVM was 2.32 and 3.53 times higher than those of CMFC and BMFC. These results indicated the bioanode and biocathode of SMFC-Biosensor together worked as sensing elements and the larger current drop was probably due to the both decreases in anodic and cathodic biofilm viabilities.

### 3.2. SMFC-Biosensor responses to OM/TA combined shocks

Acute OM pollution should be early warned as well. However, OMs served as the substrate for anodic electricigens. The positive effect of increased OMs on CMFC signal would offset the negative effect of TA, resulting in the false-negative alert of the combined shock by the bioanode-based MFC-Biosensor. In contrast, the impact of OM on SMFC signal followed a different pattern (Fig. 3a). The SMFC current continuously increased with the NaAc concentration within the range of 0.020–0.062 g/L while the NaAc in excess of 0.123 g/L suppressed the electricity generation. The increased NaAc from 0.082 g/L to 0.164 g/L reduced the SMFC current by 16% while it was 8% in BMFC (Fig. 3b). There might be two reasons accounting for SMFC larger current drop. On the one hand, the excessive OMs would compete with the cathode to serve as an additional electron donor for biocathode, deteriorating the ability to consume cathodic electrons (Jiang et al., 2017). Besides, since the sequential flowing mode would cause a cross-inoculation effect, two bacterial communities were developed on SMFC cathode: (i) aerobic heterotrophs that grew on acetate as electron donor and oxygen as electron acceptor and (ii) autotrophs that catalyzed oxygen reduction to water while receiving electrons from the cathode (Freguia et al., 2008). The excessive OMs would stimulate the activities of aerobic heterotrophs and reduced the available dissolved oxygen for cathodic autotrophs, resulting in the disequilibrium between the two bacterial communities (Xu et al., 2014). The negative effect of excessive OMs suggested the SMFC-Biosensor was probably able to alert the OM/TA combined shock and delivered higher sensitivity than BMFC.

The sensor responses to the three types of combined shocks were presented in Fig. 3c. Under the NaAc/CTC shock, both the SMFC and BMFC currents continuously decreased whereas the CMFC current increased by 5%. The SMFC current rapidly declined from 42.51 μA to 22.36 μA within 30 min and the IR was 5.04 times higher than that of BMFC. Similar results were also observed in the sensor responses to NaAc/AVM shock (Fig. 3d). This phenomenon suggested the SMFC-Biosensor could avoid false-negative testing results towards the OM/TA combined shocks and delivered higher sensitivity than BMFC. Moreover, although the IR values of SMFC to the combined shocks were more than 50%, complete recovery could be achieved within 1–2 h as the influent was switched back to fresh medium.

Compared with the responses to NaAc/CTC and NaAc/AVM shocks, the NaAc/$Hg^{2+}$ shock caused an increased IR of 61% in SMFC whereas an unexpectedly decreased IR of 4% in BMFC (Fig. 3d). This indicated the positive effect of $Hg^{2+}$ on BMFC signal due to the reaction (ii) partly offset the negative effect of NaAc, resulting in the deteriorated sensitivity. As for some heavy metal ions such as $Cr^{6+}$ and $Co^{3+}$ who had higher redox potential, it was much easier for these substances to compete with oxygen to accept cathodic electrons (Chung et al., 2016). Accordingly, the offset effect would be more significant and this might further decrease the sensor sensitivity or even resulted in a false alert of the OM/TA combined shock. Nevertheless, the SMFC sensitivity was not largely impaired by the offset effect (Fig. 3d) which would be an advantage of SMFC-Biosensor over biocathode-based MFC-Biosensor.

The small scale SMFC-Biosensor constructed in this study successfully monitored the three types of single toxic shocks and the OM/TA combined shocks. It also delivered higher sensitivity than CMFC and BMFC, verifying the feasibility and superiority of simultaneously using

bioanode and biocathode as sensing elements to monitor toxicity. Based on the effect of toxic shock on biofilm viability and the inhibitory impact of excessive OMs, the working principle of SMFC-Biosensor could be deduced. Firstly, the presence of TA in anodic chamber inhibited the anodic biofilm viability, deteriorating the ability to generate electricity and the removal rate of OM. Then, TA in anodic effluent flowed into the cathodic chamber and further inhibited the cathodic biofilm viability and the capacity of accepting electrons. Meanwhile, the excessive OMs entering into the cathodic chamber would also cause negative effects on cathodic performance as described above. These negative effects of TA and excessive OMs destroyed both the anodic and cathodic performances, resulting in the more severe output signal decay.

## 4. Conclusions

This study first reported developing SMFC-Biosensor to monitor toxicity, testifying the feasibility of simultaneously using bioanode and biocathode as sensing elements. The sensitivity of SMFC-Biosensor to single toxic shocks was over twice higher than CMFC and BMFC. Meanwhile, the complete recovery of electricity generation was achieved within 1 h after the toxic shocks. Due to the inhibitory effect of excessive OMs on biocathode, the SMFC-Biosensor could successfully monitor the OM/TA combined shocks. Future studies would detailly investigate the SMFC-biosensor sensitivity using bioelectrochemical systems in three-electrode mode, and measure the dose relationship between IR and TA as well as the sensor responses to real samples.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.01.009.

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