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# Toxicity detection in water containing heavy metal ions with a self-powered microbial fuel cell-based biosensor

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ABSTRACT

In this work, a self-powered microbial fuel cell (MFC)-based biosensor was developed for detecting toxicity in water containing heavy metal ions. Experimental conditions containing concentration of potassium ferricyanide, load resistor and glucose concentration were optimized. Six heavy metal ions (2 mg/L, $Cu^{2+}$, $Hg^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$ and $Cr^{3+}$) were tested by this biosensor and the inhibition ratios obtained were 12.56%, 13.99%, 8.81%, 9.29%, 5.59% and 1.95%, respectively. The inhibition ratios of 28.13% was also obtained for detecting the laboratory wastewater containing several heavy metal ions. The experimental results exhibited the feasibility and potential of the self-powered biosensor in detecting toxicities in water containing heavy metal ions.

## 1. Introduction

It is a large challenge that steadily and sensitively monitoring of toxicity in water due to compositions of actual water are becoming more and more complex. The design and development of biosensors towards the determination of acute total toxicity in water are of great interest due to the increasing of water pollution [1–5]. Electrochemical biosensors based on microorganism show excellent promise in toxicity determination with obvious advantages: (1) microorganism has sharp response to toxin and short life cycle; (2) electrochemical methods are sensitive and electrochemical devices are cost-effective [6,7]. The change of microorganism activity in the respiration chain is generally regarded as the response to the toxicity in such biosensors, which can be determined by electrochemically measuring the consumption of oxygen [8] or the immigrated mediator [9]. Although these biosensors show high sensitivity to various toxic chemicals, some drawbacks such as the fluctuant of oxygen concentration and potential toxicity of mediator used to microorganism should be overcome in term of practical application [10,11].

Toxicity detection biosensor for water based on electrochemically active bacteria has become the focus of attention recently [12–14]. Microbial fuel cells (MFCs) are devices that directly convert the chemical energy in organics into electricity energy via metabolic processes of microbes [15]. Mediator-less MFC had been invented by Kim et al. as a lactate biosensor in 1999 [16], the current generated from the MFC was in proportion with lactate concentration within 30 mM, which provided a possible method to monitor or detect the biofilm responses through recording of current or voltage if water quality changed. Therefore, MFCs were not only extensively applied in fields of catalytic activities of bioremediation [17] and power generation [18], but also able to used as biosensors of detecting toxicity or BOD by depending on the responses of current or voltage [19–21].

The self-powered MFC-based water toxicity detection device can be run for a long time, and it does not need a lot of maintenance, therefore, it is very suitable for online water quality monitoring [13,22]. Kim et al. utilized MFC as toxicity biosensor, and the inhibition ratios resulting from Hg, Pb, polychlorinated biphenyls (PCBs) and organophosphorus compound(OP) were 28%, 46%, 38% and 61% (1 mg/L of toxicant), respectively [23]. Comprehensive biological toxicity monitor instrument (HATOX-2000) has been applied commercially, but, the toxicity sensor system was deficient because it has large energy consumption due to needing to drum gas continuously in the cathode, oxygen was penetrated into the anode to involve the oxidize reaction, and Pt catalyst can be easily poisoned and deactivated in the microbial environment. Besides, wang et al. studied current responses on toxin concentration using formaldehyde as a toxicant over a concentration range from 0.01% to 0.10% [20] with a single chambered bioelectrochemical system (BES). The sensitivity of

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MFC-based biosensor for nickel was investigated by Hamelers et al. [24]. Deng et al. evaluated the effects of copper ($Cu^{2+}$) on soil microorganisms based on MFCs [25]. The technology may also be highly cost effective relying on the choice of manufacturing materials [26]. However, the self-powered MFC-based toxicity detection biosensor with complete response cycle has been not systematic investigated yet.

Consequently, we presented a dual-chamber self-powered MFC-based biosensor to detect toxicity in water containing heavy metal ions. Potassium ferricyanide was chosen as cathode oxidant in place of oxygen owing to its good water-soluble and high electrochemical activity of the oxidant. Experimental conditions including concentration of the potassium ferricyanide, load resistor and glucose concentration are optimized. The biotoxicity responses of heavy metal ions such as $Cu^{2+}$, $Hg^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$ and $Cr^{3+}$ were tested by the self-powered MFC-based biosensor. The results exhibited that respiration activity of electroactive biofilm in MFC can be inhibited to a certain degree in the presence of heavy metal ions.

## 2. Experimental details and methods

### 2.1. Chemicals and instruments

Potassium ferricyanide, $CdCl_2{\cdot}2.5H_2O$, $ZnSO_4$, $(CH_3COO)_2Hg$, $PbNO_3$ and $Cr(NO_3)_3{\cdot}9H_2O$ were bought from Sinopharm Chemical Reagent Beijing Co., Ltd, and $Cu(CH_3COO)_2{\cdot}H_2O$ was bought from Tianjin East China Regent Factory, laboratory wastewater containing several heavy metal ions was obtained from our laboratory. The solutions of potassium ferricyanide with desired concentrations were prepared freshly with 100 mM phosphate buffer solution (PBS, containing $Na_2HPO_4$ 9.152 g/L, $NaH_2PO_4{\cdot}H_2O$ 6.642 g/L, $NH_4Cl$ 0.62 g/L and KCl 0.26 g/L), then wrapped with aluminum foil and stored in the dark until use. Unless otherwise stated, all chemicals were of analytical grade and used as received without further purification, and all solutions were prepared using deionized water (18.25 MΩ/cm) puried with a Milli-Q Gradient System (Millipore).

Morphology and structure characterizations were obtained with an XL30E SEM field-emission scanning electron microscope (SEM) at an accelerating voltage of 15 kV. A multimeter (Model 2700, Keithley Instruments, Inc.) was used to measure the voltage (U, V) over the external resistor. The electrochemical tests were performed with an electrochemical workstation (CHI 832B, Shanghai Chen Hua Instrument Co., Ltd.) at 30 ± 1 ℃, the graphite felt was used as the working electrode, and a self-made KCl-saturated Ag/AgCl electrode and a Pt sheet were used as the reference and counter electrode, respectively. All potentials given here were versus the KCl-saturated Ag/AgCl reference electrode.

### 2.2. MFC construction

The physical diagram of the MFC-based sensor was shown in Fig. S1, the dual-chamber MFC (cylinder size of anode chamber: 2 cm×π×(1.5 cm)$^2$ with a working volume of 14.13 mL, and cylinder size of cathode chamber: 3 cm×π×(1.5 cm)$^2$ with a working volume of 21.2 mL (Fig. S1)) was constructed by using several cuboid organic glass blocks with several holes (Hong Kong Phychemi Company Limited), which were sealed with silicone gaskets and screwed to prevent leakage, anode chamber and cathode chamber were separated with a cation exchange membrane (CMI-7000, Membranes International Inc.). Two graphite felts (size: 1 cm×1 cm, thickness: 1 cm, Beijing Sanye Carbon Co., Ltd.) were used as anode and cathode electrodes with 1 cm interval, the graphite felts were pretreated by 1 M HCl overnight and washed by deionized water for three times.

### 2.3. Sludge acclimation and culture medium

The anaerobic sludge was collected from the Changchun second sewage treatment plant, located in Jilin Province, northeast China. The raw sludge was filtered with a G2 Frit funnel and acclimatized by batch mode at pH 7.0 PBS in the preparatory stage, two-thirds of supernatant was replaced by fresh medium every 2 weeks [27].

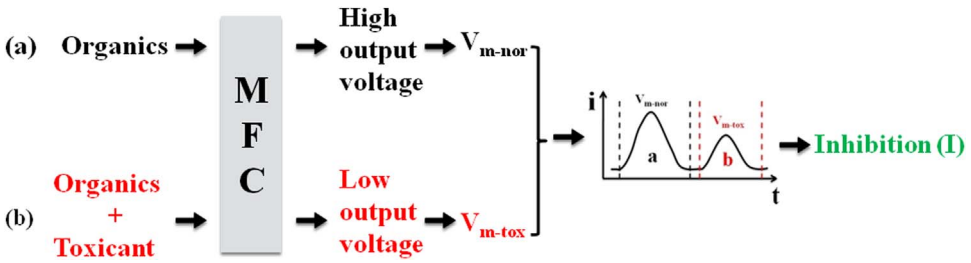
The medium contained 1 g/L glucose dissolved in 100 mM PBS amended with 12.5 mL/L minerals and 5 mL/L vitamins [28]. Minerals composed of (per liter deionized water): 1.5 g aminotriacetic acid, 3.0 g $MgSO_4$, 0.5 g $MnSO_4{\cdot}H_2O$, 1.0 g NaCl, 0.1 g $FeSO_4{\cdot}7H_2O$, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.1 g $CoCl_2{\cdot}6H_2O$, 0.13 g $ZnCl_2$, 0.01 g $CuSO_4{\cdot}5H_2O$, 0.01 g $AlK(SO_4)_2{\cdot}12H_2O$, 0.01 g $H_3BO_3$, 0.025 g $Na_2MoO_4$, 0.024 g $NiCl_2{\cdot}6H_2O$ and 0.025 g $Na_2WO_4{\cdot}2H_2O$. Vitamins (100 times) composed of (per liter deionized water): 0.2 g vitamin H, 0.2 g folic acid, 1 g vitamin B6, 0.5 g lactochrome, 0.5 g oryzanin, 0.5 g nicotinic acid, 0.5 g vitamin B5, 0.01 g vitamin B12, 0.5 g p-aminobenzoic acid and 0.5 g lipoic acid. Medium was used as nutrients in the startup phase, and used as the water sample in toxicity testing stage. Medium in the absence of glucose was used as cleaning solution in anode chamber, medium in the presence of toxic substances was used as sample solution. Potassium ferricyanide solution was used as the cathode electron acceptor. The growth mediums were sparged with $N_2$ to remove dissolved oxygen before use.

### 2.4. Startup of the MFC

The MFC was discharged under constant-loading mode by connecting anode and cathode with a fixed external resistor (1000 Ω) at a constant temperature (30 °C) in an incubator (DH 4000B II, Tianjin Taisite Instrument Co., Ltd) in the startup period. After the output voltage decreased to 5% of its peak value, the medium in anode chamber and potassium ferricyanide in cathode chamber were aspirated with a syringe, then the anode chamber was refilled with the fresh medium solution include 30% acclimated activated sludge, and the cathode chamber was refilled with 50 mM fresh potassium ferricyanide. The startup of the MFC was successful when peak voltages of two consecutive cycles stayed stable.

### 2.5. Quantization of toxicity detection

Schematic illustration of toxicity detection in water based on self-powered MFC-biosensor was shown in Scheme 1. After the successfully startup of MFC and stably output voltage signal, anode solution of the




**Scheme 1.** Schematic illustration of toxicity detection in water based on self-powered MFC.

MFC was taken out, and organic substrate with certain concentration (× mg/L) was injected, high output voltage curve (a) and the peak voltage ($V_{m\text{-}nor}$) were obtained. When the voltage dropped below 50 mV, firstly, cathode solution was replaced with 50 mm potassium ferricyanide, and then, the anode solution was taken out, organic substrate of the same concentration (× mg/L) containing a certain concentration of toxic was injected, low output voltage curve (b) and the peak voltage ($V_{m\text{-}tox}$) were obtained. The toxicity inhibition ratio (I) was calculated according to formula (1). Which can be interpreted as the respiratory and metabolic activity of microorganisms were inhibited in the presence of toxicants, resulting in less electrons produced, therefore, the output voltage became lower. Toxicity detection in water was quantitated according to the difference of the output voltages.

Toxicity can be determined by measuring changes of the maximum output voltage in a cycle. When the standard deviation of three normal maximum voltage peaks was within 5%, the toxic substances were added. The inhibition ratio (I) caused by the toxic substance were given by formula (1) [23].

$$I(\%) = 100 \times (V_{m-nor} - V_{m-tox})/V_{m-nor} \quad (1)$$

Here, $V_{m\text{-}nor}$ was the maximum output voltage resulting from glucose solution without toxic substance in the MFC anode chamber; $V_{m\text{-}tox}$ was the maximum output voltage resulting from glucose solution containing toxic substance in the MFC anode chamber.

## 3. Results and discussion

### 3.1. Startup of the MFC and its performance characterization

Graphite felts, porous carbon fiber material (Fig. 1(A)), were used as electrode material of the anode and cathode. As can be seen from the SEM image in Fig. 1(B), the graphite felt of anode electrode had been covered with biofilm after startup of MFC successfully and ran stably. Fig. S1 exhibited the picture of the dual-chamber MFC-based biosensor with a 1000 Ω external resistor. The load voltage gradually increased during the evolved process, and reached about 0.56 V after 150 h, and the maximum output voltage no longer rose in the fifth cycle, which indicating the successful startup of the MFC (Fig. S2).

To characterize the electron transfer interactions of microbial biofilms in details, by using Cyclic Voltammetry (CV) we studied biofilms on the anode (see Fig. 2(A)). The CV curve of the biofilm had two couples of redox peaks in the range of −0.39 ~ −0.24 V and −0.22 ~ −0.17 V, respectively. These results demonstrated that the biofilms exhibited a highly complex condition similar to previous reports [29], and according to the literature, the two redox peaks could be caused by electron transfer from outer membrane cytochromes. Further, Fig. 2(B) exhibited the open circuit voltage (OCV) and the power density of the evolved MFC of 0.635 V and 3.16 W/m$^2$ using linear sweep voltammetry (LSV), respectively.

### 3.2. Effect of the potassium ferricyanide concentration

As an important component of dual-chamber MFC, the cathode has a great effect on the electricity generation characteristics. Ferricyanide ($FeCN_6^{3-}$) is a negative ion complex that can take up one electron to form ferrocyanide ($FeCN_6^{4-}$), and it was chosen as the cathodic electron acceptor in this work due to several reasons as follows: (1) Ferri/ferrocyanide are known to be a very fast redox couple and the electron transfer rate is far higher than that of anode biofilm. The anode will be the rate-limiting step when ferricyanide is used as cathodic electron acceptor, therefore, and the current/potential change of MFC is determined by the anode current/potential, which demonstrated that the MFC performance can be used to detect toxicity in water; (2) ferricyanide has a low standard potential and highly soluble in water [30]; (3) ferricyanide cathode is more stable than oxygen owing to its fixed concentration, whereas oxygen concentration varies with the condition and activity of Pt catalyst is also instable. Therefore, it does not effect the stability of the whole battery when potassium ferricyanide was used as electron acceptor in the dual-chamber MFC.

In order to investigate the effect of potassium ferricyanide concentration on the maximum output voltage, 12.5, 25.0, 50.0 and 100.0 mM potassium ferricyanide were injected successively into the cathode chamber, respectively. It can be seen from Fig. 3 that the load voltages were gradually increased with increasing of concentration of potassium ferricyanide. The platform was basically reached at 50.0 mM potassium ferricyanide, the load voltage only increased 0.1 V when concentrations of potassium ferricyanide increased from 50.0 mM to 100.0 mM, in other words, the load voltage was not significantly increased when higher concentration of ferricyanide potassium was added. Furthermore, high concentration of potassium ferricyanide could be easily penetrated into the anode chamber and damaged the microorganisms inside. Therefore, 50.0 mM was chosen as the optimization concentration of potassium ferricyanide, the subsequent experiments all chose 50.0 mM potassium ferricyanide as cathodic electron acceptor.

### 3.3. Effect of the load resistor

Load resistor was also optimized because it has an important effect on the biosensor sensitivity and response speed. It was shown in Fig. 4(A), the smaller load voltage values (0.026 V, 0.126 V and 0.225 V) were obtained with lower resistors (30 Ω, 100 Ω and 200 Ω), respectively. Higher load voltage values (0.527 V and 0.544 V) were obtained with larger load resistors (620 Ω and 820 Ω), respectively, however, it needed longer time to absolutely degrade the substrate. It can be seen from Fig. 4(B) that voltages linearly varied with resistors in the scope of 30 Ω~390 Ω, but, the load voltage values increased little when the load resistor increased to 620 Ω and 820 Ω. Therefore, 390 Ω was chosen as the optimization load resistor considering both the load voltage and response time.

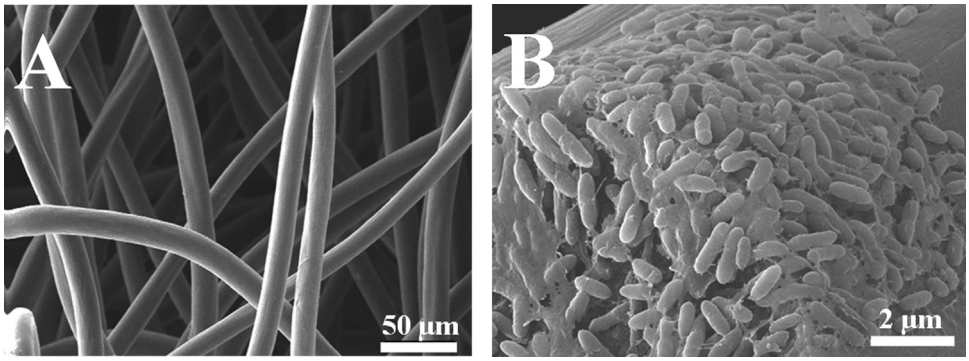



**Fig. 1.** SEM images of (A) the graphite felt and (B) the electroactive biofilm grown on the graphite felt anode.

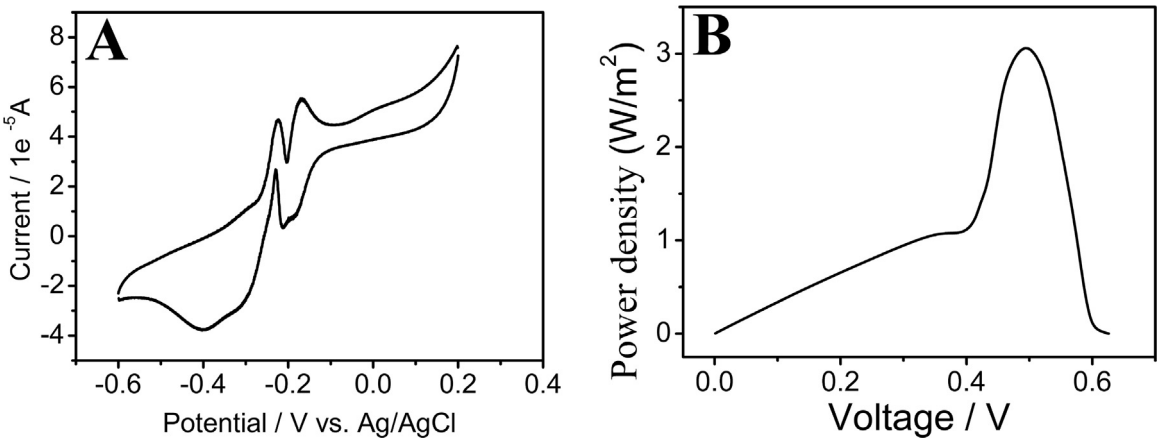



**Fig. 2.** (A) CV curve of MFC anode in PBS at a scan rate of 2 mV/s; (B) Power density curve of the evolved MFC, obtained from LSV at a scan rate of 1 mV/s .

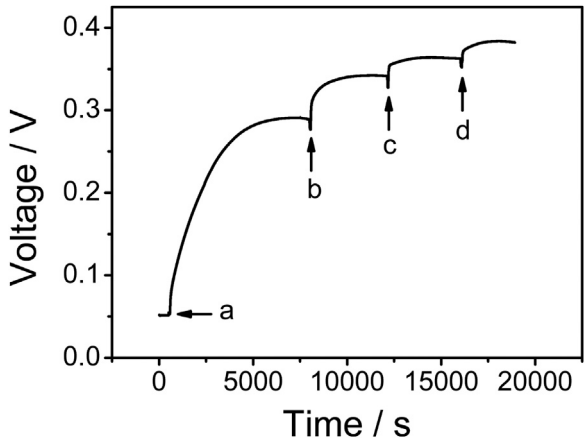



**Fig. 3.** Variation of load voltage with time at potassium ferricyanide concentration of (a) 12.5 mM, (b) 25.0 mM, (c) 50.0 mM and (d) 100.0 mM, the MFC ran under the condition of 390 Ω load resistor and 300 mg/L glucose.

### 3.4. Effect of the glucose concentration

Quick and sensitive toxicity detection was also determined by a suitable substrate concentration. As shown in Fig. 5(A), low concentrations of glucose (31.25–250 mg/L) were quickly degraded, however, the load voltages (0.038 V~0.277 V) obtained were also small. 0.396 V and 0.478 V were obtained when the concentrations of glucose were 500 mg/L and 375 mg/L, respectively. A high load voltage (0.56 V) was obtained for 1000 mg/L glucose, but it needed longer time to degrade the abundant glucose. Fig. 5(B) exhibits variation of maximum voltage with glucose concentrations, and it can be seen clearly from Fig. 5(C) that a good linear relationship ($R^2$ =0.9895) between maximum voltages and glucose concentrations in the scope of 31.25–375 mg/L glucose under 390 Ω load resistor. Load voltage increased slowly when the concentration of glucose sequentially increased, therefore, 375 mg/L was chosen as the optimization concentration in the toxicity detection. It is worth mentioning that the biosensor might be also able to detect biochemical oxygen demand in water based on the above linear relationship.

### 3.5. Toxicity tests

Heavy metal ion is one of the most serious environmental pollutant due to its toxic effect, and it can be long and large accumulated in aquatic ecosystems [31]. At present, heavy metal pollution detection in aquatic environment has become the focus of public attention in environmental science field, therefore, it is necessary to detect toxicity in water containing heavy metal ions.

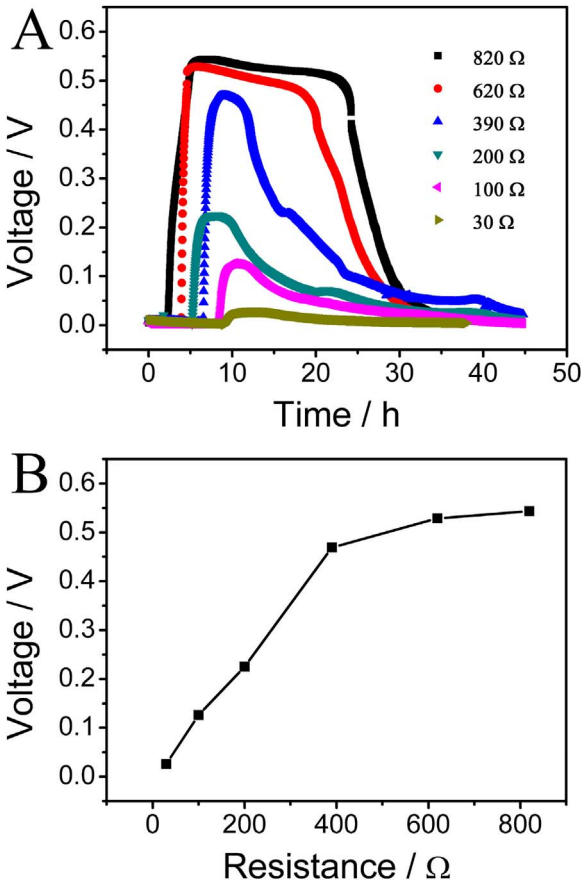



**Fig. 4.** Effect of load resistance on output voltage at 1000 mg/L glucose, (A) output voltage curve under different load resistor; (B) variation of maximum load voltages with load resistors.

In view of the fact that microorganism is very sensitive to $Cu^{2+}$(Yu et al., 2013), $Cu^{2+}$ was used as the model toxic substance in this work. We can see that there were all two peaks in both Fig. 6 and Fig. 7, in which the first peak in each graph corresponding to the output voltage of the glucose solution without toxic substance in the anode chamber, the peak voltages were $V_{m\text{-}nor}$, and the second peak in each graph corresponding to output voltage of the glucose solution containing a certain concentration of toxic substances in the anode chamber, the peak voltages were $V_{m\text{-}tox}$. $V_{m\text{-}nor}$ and $V_{m\text{-}tox}$ in Fig. 6 and Fig. 7 were summarized in Table 1, and the I values were also calculated according to the formula (1). It was obvious that Fig. 6 showed significant inhibitory effects of MFC anode microbial respiration at different

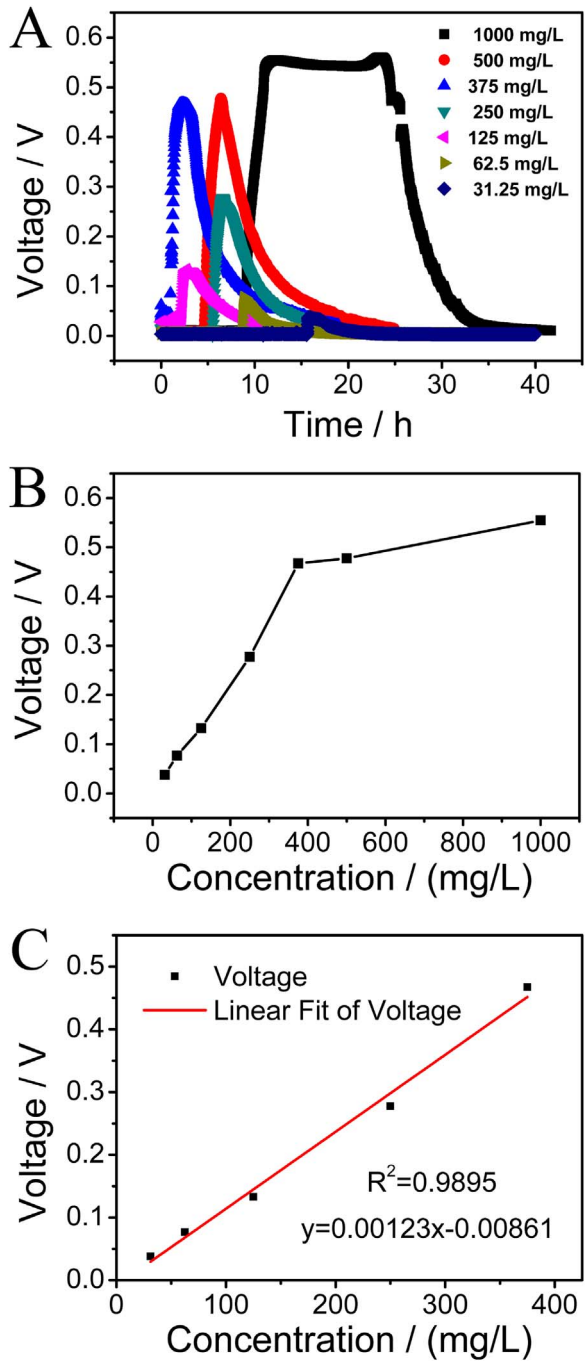



**Fig. 5.** Effect of substrate concentration on output voltage at 390 Ω load resistor, (A) output voltage curve under different concentrations of glucose; (B) variation of maximum load voltages with glucose concentrations; (C) linear relationship between maximum voltages and glucose concentrations.

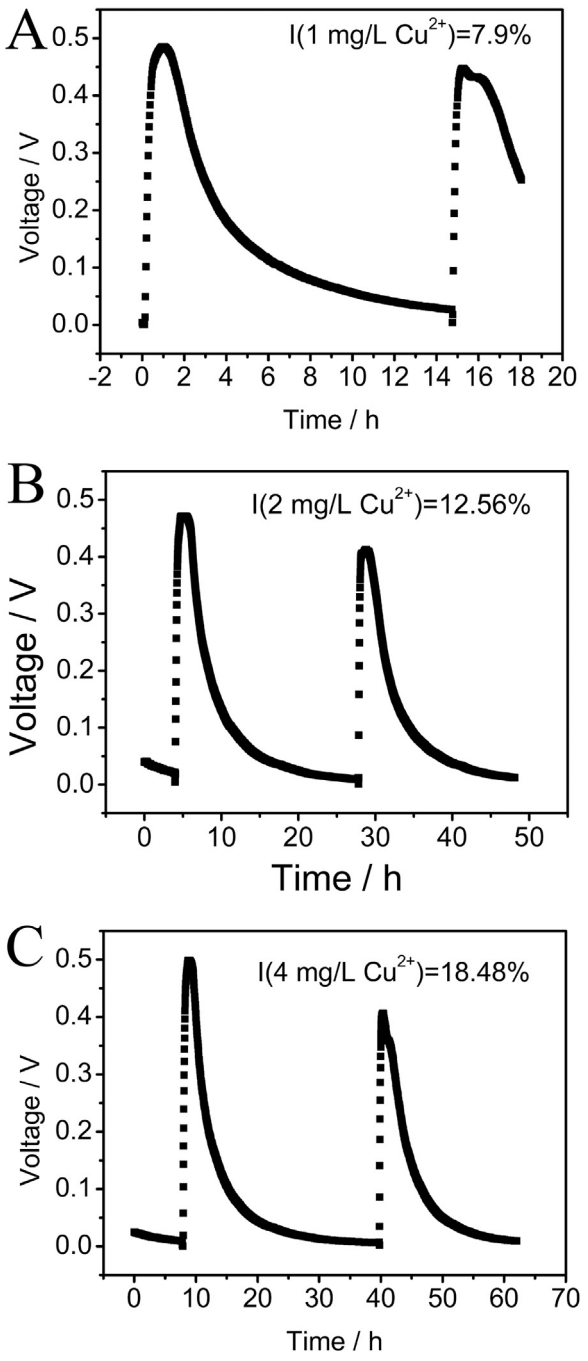



**Fig. 6.** Inhibition of microbial in the MFC anode chamber for $Cu^{2+}$ of (A) 1 mg/L, (B) 2 mg/L and (C) 4 mg/L.

concentrations of $Cu^{2+}$, and these effects were stronger with increasing of $Cu^{2+}$ concentrations. I values were 7.9%, 12.56% and 18.48% in the samples containing 1, 2 and 4 mg/L $Cu^{2+}$, respectively. Fig. 7 displayed that I values were 12.56%, 13.99%, 8.81%, 9.29%, 5.59% and 1.95%, for 2 mg/L $Cu^{2+}$, $Hg^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$ and $Cr^{3+}$ at the same concentration, respectively. The toxicity order, according to the I values for 2 mg/L heavy metal ions, is $Hg^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+} > Pb^{2+} > Cr^{3+}$, which is basically consistent with the reported toxicity order obtained using the Microtox_chronic toxicity test [32]. The marine bioluminescent bacteria, *V. fisheri*, was used as the test organism in the Microtox_chronic toxicity test, but the method was restricted by cell species strictly and wasn't suitable for samples of high turbidity. Therefore, the detection method proposed with complete working cycle has excellent promise of toxicity detection in water. Finally, we also detected the laboratory wastewater containing several heavy metal ions, and I value of 28.13% was obtained.

Inhibition of heavy metal ions to microbial respiration can be seen as inhibition to enzyme activity, which may occur in enzyme synthesis paragraph, the enzyme reaction stage and the transmission with the active enzyme or other related parts. It may lead to a result that all chains are paralyzed at degradation or metabolic reactions if enzyme activity is inhibited, resulting in the cell activity is inhibited completely, even cell died eventually. When toxicants are injected, corresponding voltages or currents will decline due to inhibition of respiratory activity, therefore, the inflow water containing toxicant is monitored by analysing changes of voltages or currents of the MFC-based biosensor.

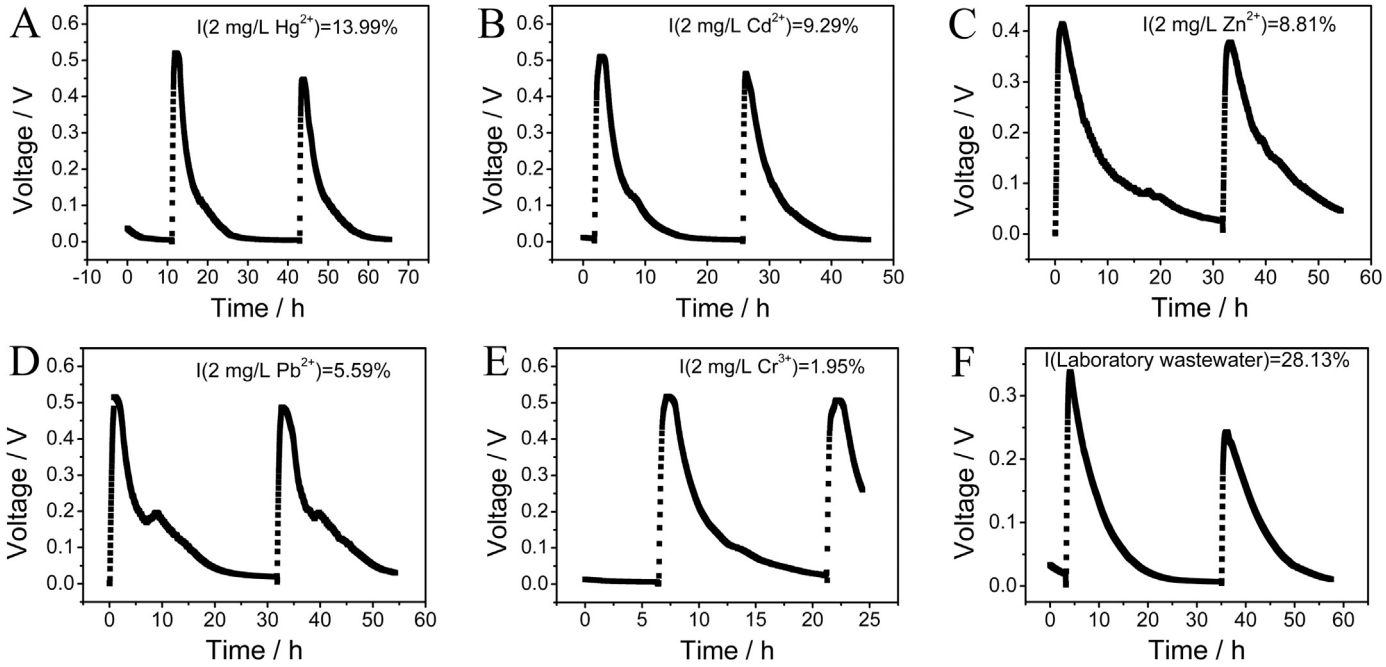



**Fig. 7.** Inhibition of microbial in the MFC anode chamber for 2 mg/L of (A) $Hg^{2+}$, (B) $Cd^{2+}$, (C) $Zn^{2+}$, (D) $Pb^{2+}$, (E) $Cr^{3+}$ and (F) Laboratory wastewater.

**Table 1**
$V_{m\text{-}nor}$, $V_{m\text{-}tox}$ and I values obtained from several heavy metal ions and laboratory wastewater with the biosensor.

| Heavy metal ions | Concentration (mg/L) | $V_{m\text{-}nor}$ (V) | $V_{m\text{-}tox}$ (V) | Inhibition ratio (I, %) |
|---|---|---|---|---|
| $Cu^{2+}$ | 1 | 0.486 | 0.447 | 7.9 |
| | 2 | 0.471 | 0.412 | 12.56 |
| | 4 | 0.499 | 0.406 | 18.48 |
| $Hg^{2+}$ | 2 | 0.520 | 0.447 | 13.99 |
| $Cd^{2+}$ | 2 | 0.510 | 0.463 | 9.29 |
| $Zn^{2+}$ | 2 | 0.414 | 0.378 | 8.81 |
| $Pb^{2+}$ | 2 | 0.516 | 0.487 | 5.59 |
| $Cr^{3+}$ | 2 | 0.518 | 0.507 | 1.95 |
| Laboratory wastewater | – | 0.338 | 0.243 | 28.13 |

—: Unknown concentration.

## 4. Conclusion

A dual-chamber self-powered MFC-based toxicity biosensor was developed and allowed detecting or monitoring of toxicity in water containing heavy metal ions. Experimental conditions containing concentration of potassium ferricyanide, load resistor and glucose concentration were optimized. $Cu^{2+}$ was chosen as a model toxicant, six heavy metal ions, such as 2 mg/L $Cu^{2+}$, $Hg^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$ and $Cr^{3+}$ were tested by this self-powered biosensor, and I values obtained were 12.56%, 13.99%, 8.81%, 9.29%, 5.59% and 1.95%, respectively. The toxicity order, according to the I values, was $Hg^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+} > Pb^{2+} > Cr^{3+}$. I value of 28.13% was also obtained by detecting the laboratory wastewater containing several heavy metal ions. The results exhibited that respiration activities of electrochemically active bacteria can be inhibited and detected sensitively in the presence of heavy metal ions. In a word, the self-powered MFC-based biosensor proposed was a simple, cheap and sensitive, and it has a excellent promise in detecting or monitoring of toxicity in water.

## Acknowledgements

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2017.03.048.

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