Assessing the Solitary and Joint Biotoxicities of Heavy Metals and Acephate Using Microbial Fuel Cell

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Abstract. A microbial fuel cell (MFC)-based biosensor can be used to detect the water toxicity. In this study, the solitary and joint biotoxicities of heavy metal ions (Cu^{2+}, Ni^{2+}, Cd^{2+}) and acephate were investigated using single-chamber MFC. The results showed that all the contaminants could individually inhibit the electricity production of MFC, and the toxicity order of these three metals was $Cd^{2+} > Ni^{2+} > Cu^{2+}$. The concentration-response curves for Cu^{2+}, Ni^{2+} and acephate were well fitted linearly while the curve for Cd^{2+} was well fitted by an exponential function. The joint-biotoxicity results of Cd^{2+}-Ni^{2+} and Cu^{2+}-acephate were as follows: the toxic effect of Cu^{2+} and Ni^{2+} was synergistic. The existence of Cu^{2+} had antagonism with acephate at low concentrations, which could reduce the biotoxicity, while their toxic effect on MFC became synergistic at high concentrations. This study could provide some basic data for water quality monitoring using MFC.

1.   Introduction

With the rapid growth of Chinese industrial capacity, the amount and diversity of water pollutants increase gradually, having created serious risks to human and environmental health [1]. Water pollution, especially acute water toxicity, has alerted people to develop more reliable and quick ways to test water quality. However, present water quality standard is incomplete and lack of sufficient ecological toxicology data. In addition, present water testing methods, which mainly include physiochemical and biological methods, could not ensure quick-test and reliable results simultaneously.

Recently, water quality monitoring technology based on microbial fuel cell (MFC) has become more and more popular because of its fast speed, small errors and capability of real-time monitoring [2]. MFC is a device which could convert biochemical energy into electrical energy using microorganisms [3]. When a toxic component appears in anolyte, it restrains the electricity production by adversely impacting the electricigen's bioactivity, thus the real-time monitoring of water quality could be realized by continuously monitoring the output voltage change of the MFC. Kim et al. invented an MFC-biosensor and found that toxic substances including Pb^{2+}, Hg^{2+} and organophosphorus compound could all inhibit MFC current, demonstrating the feasibility of detecting toxic materials by MFC [4]. In the last decade, the feasibility of using MFC to detect biotoxicity of Cu^{2+}, Ni^{2+} and HCHO have been verified respectively in many studies [5-7], and their detection limits and detection ranges have also been determined. However, few studies have been done to assess the joint toxicities of pollutants, which could better represent the common co-existence of pollutants. Therefore, study on the joint toxicities of contaminants is needed for more accurate

assessment of ecological risk. Hence, in our research, the single toxicities of heavy metals (Cu^{2+}, Ni^{2+}, Cd^{2+})and acephate which were frequently detected in the enviroment were studied firstly using single-chamber MFC (SCMFC). Furthermore, the joint toxicities of Cd^{2+}-Ni^{2+} and Cu^{2+}-acephate were also investigated and discussed. Our study will provide basic information on utilizing MFC for toxicity assessment of heavy metals and acephate.

2.   Materials and methods

2.1. Chemicals and materials

Reagent grade $CuSO_4$, $CdSO_4 \cdot 6H_2O$, $NiSO_4 \cdot 6H_2O$ were used as the heavy metal contaminants. The acephate was obtained from commercially available organophosphorus pesticides (with 3% of acephate).The heavy metals and acephate were dissolved in anolyte to make stock solutions. The concentrations of heavy metals and acephate stock solutions were as follows: 15mg/L, 45mg/L, 105mg/L, 150mg/L Cu^{2+} solutions; 1.5mg/L, 4.5mg/L, 7.5mg/L, 15mg/L Ni^{2+} solutions; 1.5mg/L, 4.5mg/L, 10.5mg/L, 15mg/L Cd^{2+} solutions; 15mg/L, 45mg/L, 75mg/L, 105mg/L acephate solutions. Furthermore, theCd^{2+}-Ni^{2+}mixture and Cu^{2+}-acephate mixture were also dissolved in anolyte with mass proportion 1:1 to make stock solutions. The concentrations of stock solutions were 3mg/L, 9mg/L, 15mg/L with Cd^{2+}-Ni^{2+} and 30mg/L, 90mg/L, 150mg/L with Cu^{2+}-acephate. All stock solutions were sealed in refrigerator at 4 °C.

The anolyte for SCMFC consisted of 0.31g/L NH_4Cl, 0.13g/L KCl, 4.09g/L Na_2HPO_4, 2.93g/L $NaH_2PO_4 \cdot H_2O$ and 0.5g/L NaAc [3], and was kept anaerobic by continuously pumping nitrogen for 3 min.

2.1. MFC construction and operation

SCMFCs were built for experiments with anode working volume of 15.0ml. Ammonia treatment carbon cloth (Q-EFB-30-075D, Nice Special Carbon Corporation, China) was used as anode. Proton exchange membrane (Nafion-117, Du Pont) was hot-pressed onto carbon paper to make a two-in-one type of cathode. The anode and cathode were connected by Ti wires and an external resistance of 500Ω was placed in between to complete the electrical circuit. All SCMFCs were inoculated with activated sludge from the anaerobic tank of a municipal wastewater treatment plant (Beijing, China) and were placed at 25°C after inoculation. A data acquisition card (USB-1608FS, Measurement Computing Corporation, USA) was used for collecting and recording the voltage every 5s.

2.2. Detection of toxicity and calculation

When the SCMFCs got stable, 5 identical ones were used to assess the single and joint toxicities of heavy metals and acephate. 1mL 15mg/L, 45mg/L, 105mg/L, 150mg/L Cu^{2+} stock solutions were injected into anode of 4 SCMFCs to simulate 1mg/L, 3mg/L, 7mg/L, 10mg/L Cu^{2+} shocks, and the other SCMFC was as a control. Similarly, 0.1mg/L, 0.3mg/L, 0.5mg/L, 0.9mg/L Ni^{2+} shocks, 0.1mg/L, 0.3mg/L, 0.7mg/L, 1mg/L Cd^{2+} shocks and 1mg/L, 3mg/L, 5mg/L, 7mg/L acephate shocks were also tested. After single toxicant tests, the Cd^{2+}-Ni^{2+} at the concentration of 0.2mg/L, 0.6mg/L, 1.0mg/L Cd^{2+}-Ni^{2+} mixture and 2mg/L, 3mg/L, 10mg/L Cu^{2+}-acephate were assessed respectively. After each injection, the output voltage of SCMFC was monitored for 4h and IR (%) was calculated according to Eq. 1, in which V_1 represented the output voltage of SCMFC at 4h after injection, V_0 represented the output voltage before injection. Then the concentration-response relationship could be described by fitting with Origin 8.0 PRO (OriginLab Corporation, Northampton, USA). Finally, The toxicities of pollutants were reflected by the index IR_{20} [9], which represented the concentration when the IR equal to 20.

$$IR(\%) = \frac{V_1}{V_0} \times 100 \;. \tag{1}$$

3. Results and discussion

3.1. Effect of toxic events

The output voltage changes of SCMFCs to detect different Cu^{2+} concentration were recorded and shown in Fig. 1. The voltage decreased sharply when $CuSO_4$ solution was injected to anode chambers and the drop rate increased with the increase of Cu^{2+} concentration. The result was consistent with previous study [4]. It was also the same as the results when using Cd^{2+}, Ni^{2+} and acephate as toxicants.
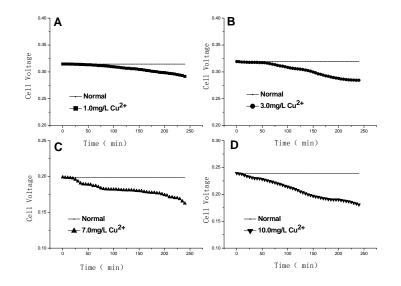

Fig. 1 The output voltage changes of SCMFCs at different Cu^{2+} shocks(A.1mg/L Cu^{2+}; B.3mg/L Cu^{2+}; C.7mg/L Cu^{2+}; D.10mg/L Cu^{2+})

3.2. The biotoxicity analysis of solitary contaminants

The voltages of SCMFCs dropped sharply after each injection of $CuSO_4$, $CdSO_4$, $NiSO_4$ or acephate solutions, which showed that all of them had biotoxicities. Fig. 2 was the concentration-response curves representing the relationship between IR and pollutant concentrations. A good linear relationship between IR and Cu^{2+} concentration was found when the concentration of Cu^{2+} was between 1.0~10.0mg/L. The concentration-response curve of Cu^{2+} was linearly fitted and was shown in Fig. 2A. The fitting equation was Y=4.87+2.08X, where Y represented IR and X was the concentration of Cu^{2+}, and the correlation coefficient (R^2) was 0.995.

Fig. 2B showed the IR of Cd^{2+} with the concentrations of 0.1mg/L, 0.3mg/L, 0.7mg/L and 1.0mg/L. It did not show a linear relationship between IR and Cd^{2+} concentration, which was similar to the previous research [10]. Therefore, we used non-linear fitting to get the concentration-response curve. The results showed the data were well fitted by an exponential function with R^2 of 0.998. The fitting equation was Y=0.0245*exp(X/0.132) +10.393, and the detected range was between 0.1 and 1.0mg/L.

Fig. 2C showed the relationship between IR and Ni^{2+} concentration. Nienke et al. have found there was a poor linear relationship between IR and Ni^{2+} concentration [6]. However, the IR of Ni^{2+} had a good linear relationship with concentration in our experiment. That might because lower concentrations in our study. The fitting equation for concentration-response curve of Ni^{2+} was Y=2.21+8.42X. The R^2 was 0.965 and the linear range was from 0.1 to 1.0mg/L.

Fig. 2D showed the concentration-response of acephate. There was also a good linear relationship between IR and concentration. The fitting equation was Y=7.74+0.80X, in which X ranged 1.0 to 7.0mg/L.

According to the fitting equation, the IR_{20} of Cu^{2+}, Cd^{2+}, Ni^{2+} and acephate were 7.27, 0.77, 2.11 and 15.33mg/L, respectively. However, the IR_{20} of Ni^{2+} and acephate exceeded their detection ranges. So we tested 2.11mg/L Ni^{2+} shock and 15.33mg/L acephate shock, the IR was 20.4 and 31.5 respectively. So the IR_{20} of Ni^{2+} was about 2.11mg/L. This demonstrated that the biotoxicities of

three metals were in the following sequence: $Cd^{2+} >Ni^{2+} > Cu^{2+}$. Gomes et al. studied the transcriptional response of E. albidus to four metals (Cu^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+}) and found the EC_{50} of four metals followed this sequence: $Cu^{2+}>Ni^{2+}>Zn^{2+}>Cd^{2+}$ [11], which was the same as our results.
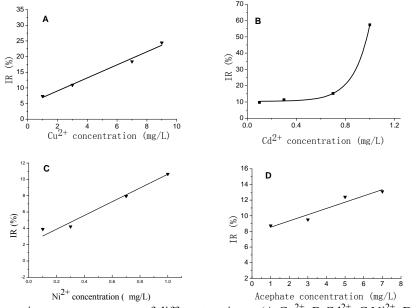

Fig. 2 The concentration-response curves of different toxicants(A.Cu^{2+}; B.Cd^{2+}; C.Ni^{2+}; D.acephate)

3.3. Joint biotoxicities of multi-pollutant

3.3.1 Detection of Cd^{2+}-Ni^{2+} pollutants

Similar to simple pollutant detection, the SCMFCs were used to detect the mixture of Cd^{2+}-Ni^{2+} at different concentrations. The evaluation of joint toxicity of Cd^{2+}-Ni^{2+} was listed in Table 1. As shown in Table 1, all the IRs of Cd^{2+}-Ni^{2+} were higher than the sum of IRs of the individual chemicals with the same concentrations as in the mixtures. This suggested that the effect of Cd^{2+} and Ni^{2+} was synergistic. Andresen et al. have found the coexistence of Cd^{2+} and Ni^{2+} was synergetic when using Ceratophyllum demersum [12], which was same as our results. In addition, Cd^{2+} did not show a good linear relationship with IR in single toxicant experiments. However, there was a good linear relationship between the concentration of Cd^{2+}-Ni^{2+} mixture and IR. The equation of fitted curve was $Y=11.25+22.25X$, where R^2 was 0.993 and X ranged from 0.2mg/L to 1.0mg/L.

Table1 joint toxicity of Cd^{2+}-Ni^{2+}

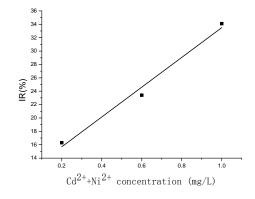| Concentration (mg/L)			IR (%)			Joint effect
Cd^{2+}	Ni^{2+}	Cd^{2+}-Ni^{2+}	Cd^{2+}	Ni^{2+}	Cd^{2+}-Ni^{2+}	
0.1	0.1	0.2	9.7	3.9	16.3	synergism
0.3	0.3	0.6	11.4	4.2	23.4	synergism
0.5	0.5	1	11.5	5.6	34.1	synergism

Fig.3 The concentration-response curve of Cd^{2+}-Ni^{2+} mixture

3.3.2 Detection of acephate-Cu^{2+} mixture

The evaluation of joint toxicity of acephate-Cu^{2+} was listed in Table 2. As shown in Table 2, the IR of mixture at 2mg/L was smaller than the sum of IRs of individual chemicals. It indicated that the coexistence of acephate-Cu^{2+} was antagonistic. However, when the concentration of mixture increased, the effect of acephate and Cu^{2+} became synergistic. It indicated that the effect of single pollutant might alter with the concentration. A good linear relationship between the mixture concentration and IR and the equation was Y=11.63+2.06X, in which X was between 2.0~10.0mg/L.

Table2 joint toxicity of Cu^{2+}-acephate

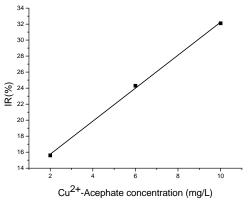| Concentration (mg/L)			IR (%)			Joint effect
Cu^{2+}	acephate	Cu^{2+}-acephate	Cu^{2+}	acephate	Cu^{2+}-acephate	
1	1	2	7.3	8.7	15.6	antagonism
3	3	6	10.9	9.5	24.3	synergism
5	5	10	15.3	12.3	32.1	synergism

Fig.5 The concentration-response curve of Cu^{2+}-acephate mixture

4. Conclusions:

(1) Cu^{2+}, Cd^{2+}, Ni^{2+} and acephate could all inhibit the electricity production of MFC, and the IR increased with the increase of the pollutant concentration.

(2) When the detection time was 4h, the equations of concentration-response curves of Cu^{2+}, Cd^{2+}, Ni^{2+} and acephate were as follows:
Cu^{2+}: Y=4.87+2.08X (1.0~10.0 mg/L);
Cd^{2+}: Y=0.025*exp(X/0.13)+10.39 (0.1~1.0mg/L);
Ni^{2+}: Y=0.68*exp(X/0.37)+2.91 (0.1~0.9mg/L);
acephate: Y=7.74+0.80X (1.0-7.0mg/L).

(3) The results of joint toxicity of binary mixture pollutants with mass proportion 1:1 showed: the effect of Cd^{2+} and Ni^{2+} was synergistic between 0.2~1.0mg/L; the coexistence of Cu^{2+} and acephate was antagonistic at 2mg/L while was synergistic at 6mg/L and 10mg/L.

(4) A good linear relationship was found between the mixture concentration and IR. The equation of concentration-response curves were as follows:
Cd^{2+}-Ni^{2+}: Y=11.25+22.25X (0.2~1.0mg/L)
Cu^{2+}-acephate: Y=11.63+2.06X (2.0~10.0mg/L)

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