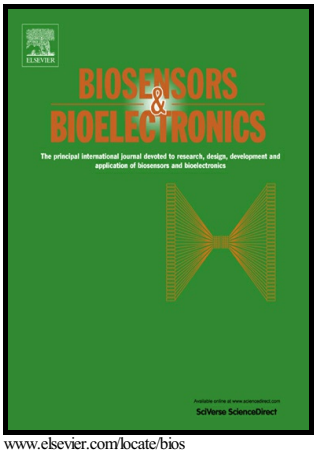

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Problems Analysis and New Fabrication Strategies of Mediated Electrochemical Biosensors for Wastewater Toxicity Assessment

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Abstract

Conventional mediated electrochemical biosensors for toxicity assessment were almost based on 'one-pot' principle, i.e., mediators and the under-test chemicals were mixed together in the same vessel. In this process, the electron mediator is assumed to be merely an electron acceptor and cannot react with under-test toxicants. Actually, some under-test pollutants (such as metal ions) could react with the electron mediators, thus affecting the detection accuracy and sensitivity of the sensors. It was also found that at least two other interference factors have been ignored in present 'one-pot' mediated electrochemical biosensor systems, i.e., (1) the electrochemical sensitivity of mediators to pH; and (2) the potential reactions between under-test chemicals and buffers and the consequent pH changes. In this study, the three ignored interference factors have been investigated systematically and demonstrated by significance tests. Moreover, a solving strategy, an isolation method, is proposed for fabrication of novel mediated electrochemical biosensor to avoid the interference factors existing at present mediated electrochemical biosensor. According to the testing results obtained from the isolation method, IC_{50} values of Cu^{2+}, Cd^{2+}, Zn^{2+}, Fe^{3+}, Ni^{2+} and Cr^{3+} were

[1] Yajie Yang and Deyu Fang contributed equally to this work.

21.3 mg/L, 3.7 mg/L, 26.7 mg/L, 4.4 mg/L and 10.7 mg/L, respectively. The detection results of four real water samples also suggested this method could be applied for the practical and complex samples.

Keywords

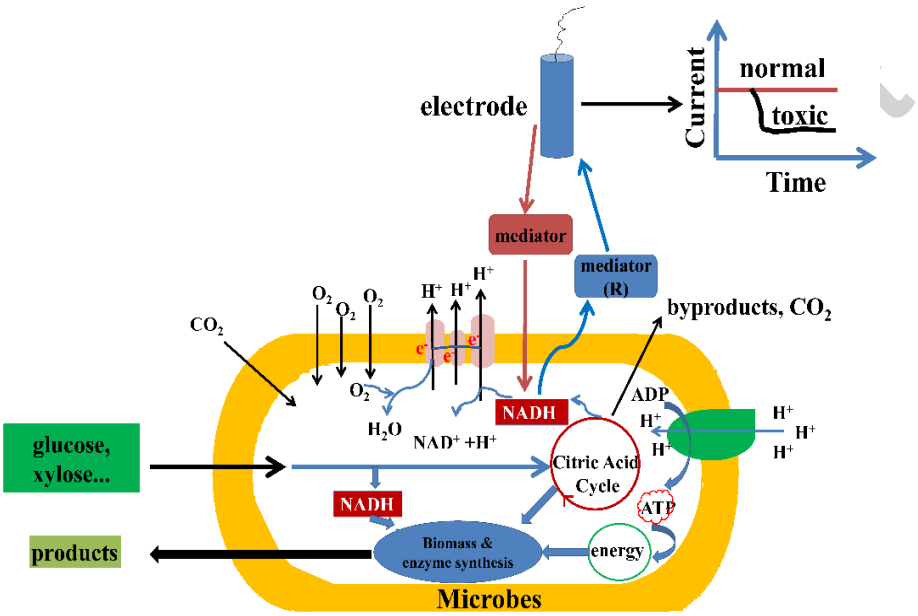
Mediated Electrochemical Biosensors; Wastewater Toxicity Assessment; Interference Factors.

1. Introduction

The rapid industrial development and widely chemical material supply produced amount of pollutions lead to increase potential risk to human health and contamination of the environment, so it is instant to detect toxicity of pollutants. Although conventional analytical methods (e.g. gas chromatography and high pressure liquid chromatography) determine specific pollutants precisely, it is incapable to reflect the environmental risks of chemicals to living organisms because toxicity is biological response (Wang et al. 2013; Pujol-Vila et al. 2015). Thus, developing effective methods of evaluating the toxicity of chemicals are significant. The past several decades have witnessed the boom of biological assays from different trophic levels, such as fish (van der Schalie et al. 2001; Polak et al. 1996), plants (Steinkellner et al. 1998; Campas et al. 2008), invertebrates (Xu et al. 2011; Tsopela et al. 2014), and microbes (Gernaey et al. 1997; Girotti et al. 2008). Among these organisms, the ubiquitous microbes were proposed and had tremendous influence on toxicity evaluation of chemicals in recent years owing to their rapid response to toxicants, short life cycle and low cost. (Bitton 2005; Dannat et al. 2003; Xiao et al. 2015).

Mediated electrochemical microbial biosensors for toxicity assessment have drawn much attention due to their easy miniaturization, robustness and low cost (Pasco et al. 2001; Liu et al. 2009; Wang et al. 2008; Li et al. 2014). Electron mediators, such as neutral red, benzoquinone, ferricyanide, etc., can intervene in the microbial respiration process and accept electrons from the electron transport chain

(ETC) to form the reduced mediators, which can be oxidized at proper potential to generate an oxidation current. The existence of toxic chemicals inhibits the microbial respiration thus producing less reduced mediators and smaller oxidation currents (Scheme 1), then according to the changes of oxidation currents, the toxicity of chemicals can be determined. In this theory, the electron mediator is assumed to be merely an electron acceptor and cannot react with under-test toxicants. The present mediated electrochemical biosensors for toxicity assessment of chemicals are all constructed based on this principle.



Scheme 1. The principle of mediated electrochemical microbial biosensors for toxicity assessment.

In general, two strategies are commonly utilized in the present mediated electrochemical microbial biosensors for toxicity assessment: (1) microbes, mediators and the under-test chemicals are mixed together and incubated for periodic times, then the supernatant solutions are collected for chronoamperometry test to determine the reduced redox mediators (Pasco et al. 2001; Liu et al. 2009; Ma et al. 2016); (2) mediators are added to microbes suspensions under chronoamperometry to generate a high steady state current, and then the under-test chemicals are added (Wang et al. 2013; Farre et al. 2001; Li et al. 2013). Although there are some differences in these two experimental procedures, both could be concluded to the 'one-pot' method, *i.e.*,

microbes, mediators and under-test chemicals are encountered directly in a system. To date, all the reported mediated electrochemical microbial biosensors for toxicity assessment are all designed based on this ‘one-pot’ principle.

However, it is well known that some metal ions presented in wastewater can react with the mediators, thus affecting the accuracy and sensitivity of the sensors. What's more, according to the characteristics of biological toxicity assessment methods, it is impossible to obtain the exact species of metal ions in the under-test wastewater timely, that is to say, we cannot choose the appropriate mediator to avoid the interference caused by these reactions. In addition, at least two other interference factors have been ignored in present mediated electrochemical biosensor systems, including (1) the electrochemical sensitivity of mediators to pH, (2) the potential reactions between under-test chemicals and buffers and the consequent pH changes. These three ignored interference factors stem from the ‘one-pot’ design concept, which under-test chemicals are directly mixed with mediators or buffers.

In this work, three kinds of interference factors in the present mediated electrochemical microbial biosensors for toxicity assessment are thorough studied by using heavy metal ions. There are three interference factors influencing accuracy of toxicity assessment results: (1) the potential reactions between under-test chemicals and mediators; (2) the electrochemical sensitivity of mediators to pH; (3) the potential reactions between under-test chemicals and buffers. Based on the systemically analysis of these problems faced by present electrochemical toxicity biosensors, a solving strategy (an isolation method) is proposed, which not only overcome the interference problems in the present electrochemical toxicity biosensors, but also improve the sensitivity and practicability of the mediated electrochemical microbial biosensors.

2. Materials and methods

2.1. Materials

Menadione, *p*-benzoquione, ferrocenemethanol (FcMeOH) were purchased from

Adamas-beta. Potassium ferricyanideis from Acros Organics. Thionine acetate was obtained from Alfa Aesa. Other used chemical reagents were purchased from Beijing Lanyi Chemical Products Co., Ltd., China. 0.1 M phosphate buffer (PB) solution with pH=7.0 was prepared by mixing 0.1 M Na_2HPO_4•$12H_2O$ and 0.1 M NaH_2PO_4•$2H_2O$ together. The pH value was adjusted to 7.0 by adding 1 M NaOH or 1 M HCl. The Lysogeny Broth (LB) (10 g/L peptone, 3 g/L beef extract, 5g/L NaCl) for *E. coli* cultivation was adjusted to pH =7.3-7.5 with NaOH (2 mol/L) and sterilized by autoclaving. The respiration substrate (RS) solution was prepared by mixing glucose, sodium succinate and sodium lactate with each of 10 mM in PB solution (pH=7.0, 0.1 M). In this study, all chemicals were of analytical grade and all solutions were prepared with Milli-Q water.

2.2. Instruments

The optical density (OD_{600}) of *E. coli* suspension was measured by a UV spectrophotometer (SECOMAM UVIKONXL) at 600 nm. All electrochemical characterizations were performed using the electrochemical workstation Princeton 263A (Princeton Applied Research, USA).

2.3. Preparation of *E. coli*

E. coli (ATCC 25922) was supplied by China General Microbiological Culture Collection Center (CGMCC) and replanted regularly on nutrient agar plates to ensure its viability. *E. coli* was cultured aerobically at 37 ℃ for 16 h. The bacteria were harvested by centrifuging at 6000 rpm for 5 min at room temperature. The concentration of bacterial suspension was set to be an optical density of 2.3 at 600 nm.

2.4. Toxicity assessment by the isolation method

The procedure of toxicity assessment by isolation method was shown in Scheme S1. First, 1mL of *E. coli* suspension (OD_{600}=2.5), 1 mL of LB and 2 mL of toxicants solution were thoroughly mixed and incubated at 37 ℃ for 1 h. Then, the solutions were centrifuged at 5000 rpm for 5 min and washed twice with deionized water. After

that, 10 mL of solution containing glucose (10 mM), sodium succinate (10 mM) and potassium ferricyanide (45 mM) was added to the washed *E. coli* and then incubated at 37℃ again for 1 h. Finally, centrifuged at 6000 rpm for 5min and obtained supernatant solutions as the measured liquid. The concentrations of ferrocyanide in the supernatant solutions were determined by chronoamperometry (0.5 V *vs.* Ag/AgCl) under continuous and constant magnetic stirring. The inhibition ratio is adopted to evaluate the toxicity of heavy metal ions and calculated according to Equation 1:

$$\text{Inhibition (\%)} = (1-i_e/i_c)\times 100\% \quad (1)$$

where i_e is the steady current in the experimental groups, i_c is the steady current in the control group. The reliability of three proposed interference factors was examined by Kolmogorov-Smirnov normality test, t-test, Kruskal-Wallis test and one-way ANOVA to analyze their normal distribution and significant difference.

3. Results and discussion

3.1. Reaction between mediators and typical heavy metal ions on toxicity assessment

Microbial respiration processes are accompanied with electron transferring, in which many electron carriers exist, such as NAD^+/NADH, FAD^+/FADH, cytochrome *b* (+3)/cytochrome *b* (+2), ubiquinone (ox)/ubiquinone (red) and cytochrome *c* (+3)/cytochrome *c* (+2) (Pasco et al. 2005; Kracke et al. 2015). The mediators have been widely used in microbial fuel cells (MFCs) and electrochemical microbial biosensors to facilitate the electron transfer process (Schaetzle et al. 2008; Lei et al. 2006). In electrochemical microbial biosensors for toxicity assessment, mediators act as electron acceptors to compete with the dissolved O_2 to accept electrons from electron carriers in ETC and then transform the microbial respiration signal to electrochemical signals.

To study the effect of mediators on toxicity assessment of heavy metal ions, six widely used mediators (neutral red, menadione, thionine, *p*-BQ, ferricyanide, FcMeOH) and five typical heavy metal ions (Cd^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Fe^{3+}) were

selected. In terms of the redox potentials of electron carriers in ETC, menadione, thionine, *p*-BQ, and so on are capable of mediating electron carriers' oxidation in theory (Fig. S1). Actually, a rising current can be observed indeed when each one of neutral red, menadione, thionine, *p*-BQ, ferricyanide or FcMeOH was added into *E. coli* suspension, suggesting that all these mediators could be used to shuttle electrons from *E. coli* respiration process to electrode (Fig. S2). Moreover, the higher redox potential of the mediators, the higher steady current can be achieved at the same concentration of mediators, i.e., FcMeOH showed the highest steady current among all mediators used. Besides, due to *p*-BQ is more hydrophobic than ferricyanide, the steady current was obtained by adding ferricyanide as the mediator is lower than that of *p*-BQ, although the redox potential of ferricyanide (0.43 V *vs.* NHE) is higher than that of *p*-BQ (0.28 V *vs.* NHE). All these results suggest we should consider mediators' redox potentials and hydrophobic degree when choosing mediators for toxicity assessment.

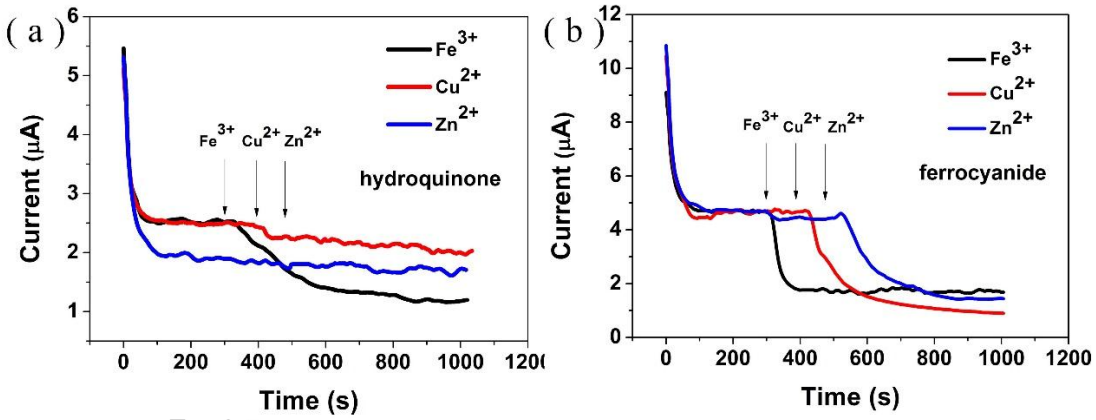


Fig. 1. Evidence of reactions between heavy metal ions and mediators: (a) hydroquinone, the reduced product of *p*-BQ; (b) ferrocyanide, the reduced product of ferricyanide. Both the concentration of hydroquinone and ferrocyanide was 2mM. The chronoamperometry were performed at 0.3 V vs. Ag/AgCl under continuous and constant stir. Fe^{3+} (black line), Cu^{2+} (red line), Zn^{2+} (blue line) were added at 300 s, 400 s, 500 s, respectively.

As mentioned above, mediated electrochemical microbial biosensors are according to the oxidation current of the mediators that reduced by microbes for toxicity assessment. More importantly, these biosensors are based on the assumption that the reduced mediators are only oxidized by the electrode and no reaction happens

between mediators and under-test chemicals. However, during actual measurement, the redox potentials of mediators and heavy metal ions were much overlapped, indicating mediators may react directly with some metal ions in the water. For example, the redox potentials of Fe^{3+}/Fe^{2+} and Cu^{2+}/Cu are 0.77 V and 0.34 V respectively, both are higher than *p*-BQ/hydroquinone (0.28 V). It means its reduced product can be oxidized by Cu^{2+} or Fe^{3+} when choose *p*-BQ as the mediator. As a result, it must influence its accuracy of the electrochemical biosensor. Moreover, it is well known that many heavy metal ions react with ferricyanide to form the Turnbull's blue analogues, or with ferrocyanide to form the Prussian blue analogues (Yu et al. 2013; Moritomo and Tanaka 2013). These reactions can be reflected by the color differences and the results are shown in Fig. S3. To test the influence of reactions between mediators and heavy metal ions for toxicity assessment, three heavy metal ions (Fe^{3+}, Cu^{2+}, Zn^{2+}) with different redox potentials were added into the hydroquinone solution and ferrocyanide solution under chronoamperometry respectively, we have compared the current before and after the metal ions added (Table S2) , it can be found that there was a decrease in the steady current when Fe^{3+} (p value < 0.0001) or Cu^{2+} (p value < 0.0001) was added into the hydroquinone solution, while the current can be stable in the case of injecting Zn^{2+} (Fig. 1a). Because the redox potentials of Fe^{3+}/Fe^{2+} and Cu^{2+}/Cu are higher than that of *p*-BQ/hydroquinone and part of hydroquinone molecules are oxidized by Fe^{3+} or Cu^{2+}, whereas the redox potential of Zn^{2+}/Zn is much lower than *p*-BQ/hydroquinone's result in no electron transform. It is worth noting that due to the redox potential of Fe^{3+}/Fe^{2+} (0.77 V) is higher than that of Cu^{2+}/Cu (0.34 V), the decrease of current with the adding of Fe^{3+} was more than that with the adding of Cu^{2+}. In addition, another mediator, ferrocyanide can coordinate with Fe^{3+}, Cu^{2+} or Zn^{2+} which cause all the steady currents to decrease dramatically (p value < 0.0001) when ions was added (Fig. 1b). Obviously, no matter strategy 1 or strategy 2 was utilized for toxicity assessment, the toxicities of Fe^{3+} and Cu^{2+} may be amplified when *p*-BQ was used as the mediator, while all toxicities of Fe^{3+}, Cu^{2+} and Zn^{2+} may also be amplified when ferricyanide was used as the mediator. All these results suggest that the potential

reactions between mediators and under-test chemicals may influence the current signals, resulting in inaccurate toxicity results. More importantly, due to the characteristics of biological toxicity assessment methods, it is impossible to obtain the exact species of metal ions in wastewater timely, so we cannot choose the appropriate mediator to avoid the interferences caused by these reactions.

3.2. Effect of pH for electrochemical behaviors of mediators

In mediated electrochemical microbial biosensors for toxicity assessment, mediators are necessities for the transformation of microbial respiration signal to current signal. Previous studies were focusing on the mediators' lipophilic or hydrophilic property on toxicity evaluation (Li et al. 2013; Matsumae et al. 2014), but the effect of pH on the electrochemical behaviors of mediators was ignored. Fig. 2 exhibits the cyclic voltammetry behaviors of neutral red, menadione, thionine, *p*-BQ, ferricynaide and FcMeOH in different pH conditions. It can be seen that the redox peaks of neutral red, menadione, thionine and *p*-BQ shift positively with the rise of pH from 5.0 to 9.0 owing to participation of hydrogen ions in their redox reaction (p value < 0.0001), while the redox peaks of ferricyanide and FcMeOH remain stable with the change of pH because their redox reactions are independent of hydrogen ions or hydroxyl ions (Table S1, S3). Moreover, the peak currents also alter with various pH in these six cases, so it is essential to maintain the solution's pH constant in the experimental process when using current signals for toxicity assessment.

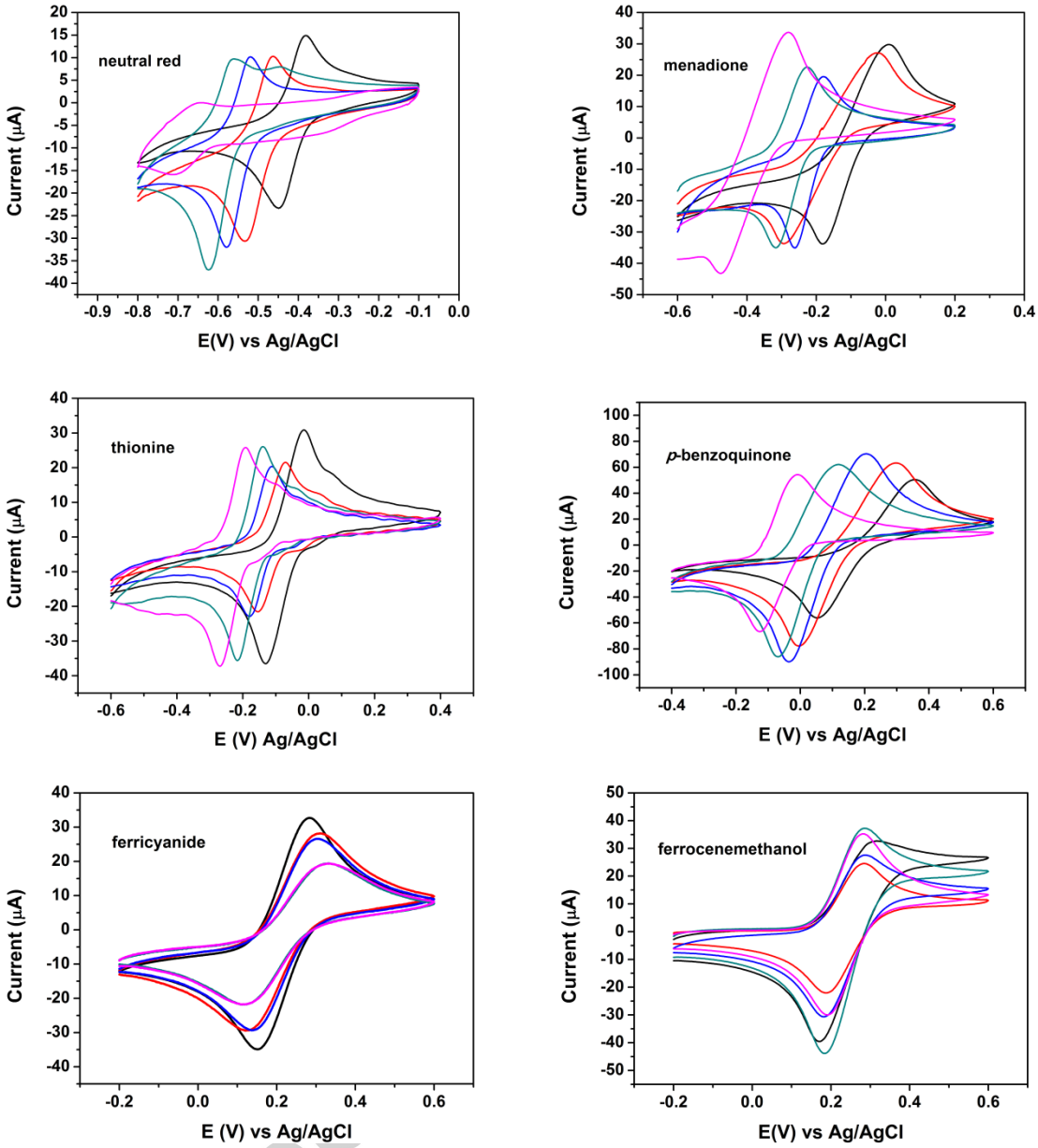


Fig. 2. The cyclic voltammetry behaviors of neutral red, menadione, thionine, *p*-BQ, ferricynaide and FcMeOH (2 mM) at pH=5.0 (magenta line), 6.0 (cyan line), 7.0 (blue line), 8.0 (red line), 9.0 (black line).

3.3. Effect of reactions between heavy metal ions and pH buffers on toxicity assessment of heavy metal ions

Buffer solutions have been widely used to keep pH at nearly constant value in many fields such as analytical chemistry, biochemistry, fermentation, et.al. Controlling pH is also critical in toxicity assessment because the toxicity of chemical may relate to pH. In the example of heavy metal, Ag nanoparticles shows a stronger cytotoxic effect on algal cells of *C. acidophila* under acidic (pH=4.0) compared to

neutral condition (pH=7.0) due to more Ag^+ ions are released under acidic condition(Oukarroum et al. 2014), and the 72-h EC_{50} value of copper to *Chlorella sp.* increase from 1.5 μg/L to 35μg/L as the pH decrease from 6.5 to 5.7 (Franklin et al. 2000), etc. Phosphate buffer (PB) or Phosphate buffer saline (PBS), mainly consisting of a mixture of monobasic dihydrogen phosphate and dibasic monohydrogen phosphate, are the most widely used buffer in the reported mediated electrochemical microbial biosensors for toxicity assessment, including toxicity evaluation of heavy metal ions, due to their low cost and easy preparation. However, as shown in Fig. 3a, most of heavy metal ions such as Zn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+} and Ag^+ reacted with dibasic monohydrogen phosphate to form precipitates. At the same time, a significant decrease (p value < 0.0001) of pH value can be detected when these heavy metal ions are added to the PB solutions, as shown in Fig. 3b and Table S4. Obviously, the precipitates formed from the heavy metal ions and dibasic monohydrogen phosphate not only damage the buffering capability of PB or PBS, but also decline the concentration of heavy metal ions in solution with the result that the actual concentration of heavy metal ions was less than the expected results. Moreover, we have mentioned many mediators such as thionine, *p*-BQ are sensitive to pH, the changes of pH resulting from the direct mixture of heavy metal ions and PB or PBS would interfere the current signals and cause inaccurate toxicity results. In the terms of unknown water waste, it is impossible to choose the appropriate buffer and mediators according to ingredients and to avoid the reaction as we mentioned above.

Since introduced by Norman Good and his coworkers in 1966 (Good et al. 1966), the Good's buffers have been extensively used in biological and biochemical research because of their excellent characteristics such as high solubility, convenient pKa values (between 6 to 8) and weak-to nonexistent complexation of heavy metal ions. As a result, it seems that Good's buffers are more suitable than the PB or PBS for mediated electrochemical microbial biosensors for toxicity assessment of heavy metal ions. Among all the Good's buffers, MOPS (3-Morpholinopropane-1-sulfonic acid) is widely recognized as negligible metal binding and recommended for metal speciation

(Good and Izawa 1972; Mash et al. 2003). The performance of using MOPS as the buffer for toxicity assessment of heavy metal ions is shown in Fig. S4, significant precipitants formed when added Fe^{3+}, Cu^{2+} or Pb^{2+} in MOPS solutions, and it was similar to PB solutions to happen pH declination. The pH values of all these MOPS solutions dropped from the initial 7.40 to 6.18-6.85. Therefore, it is impossible to avoid the overall interactions between heavy metal ions and buffers just by choosing different buffers.

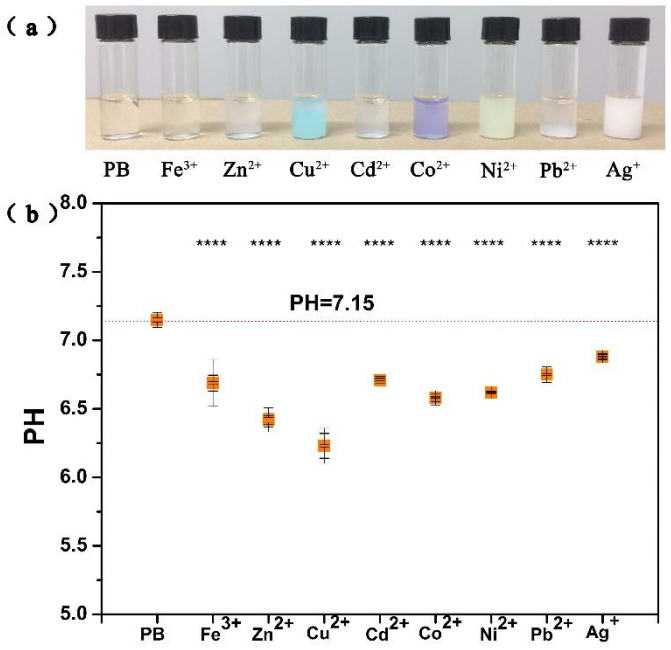


Fig. 3. Colors of pure PB and the mixtures of PB with various heavy metal ions (Fe^{3+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Ag^{+}). The mixtures are obtained by mixing 1 mL of PB solution (100 mM) with 1 mL of heavy metal ion solution (1000 mg/L).

4. Evaluating the toxicity of heavy metal ions using new mediated biosensor constructed by an isolation method

As mentioned above, there exist three interferences in the reported mediated electrochemical microbial biosensors for toxicity assessment of heavy metal ions: (1) the potential reactions between heavy metal ions and mediators; (2) the electrochemical sensitivity of most mediators to pH; (3) the potential reactions between heavy metal ions and buffers and the consequent pH changes. To our best knowledge, it is the first time to summarize these interferences. It is not difficult to

find that the origin of these interferences is the direct contact of heavy metal ions with mediators or buffers. As a result, only avoiding the direct contact of heavy metal ions with mediators or buffers can eliminate the interferences and make sure the accuracy of the toxicity assessment results. Based on above analysis, we propose an isolation method to supply the gaps. As described in Scheme S1, the under-test chemicals were mixed with microbes first and incubated for some time, then centrifuged the mixture to clear out the under-test chemicals. After that, microbes were mixed with mediators and incubated for some time. Finally, collected for electrochemical analysis at proper potential and the obtained oxidation currents of the reduced mediators were used for toxicity assessment.

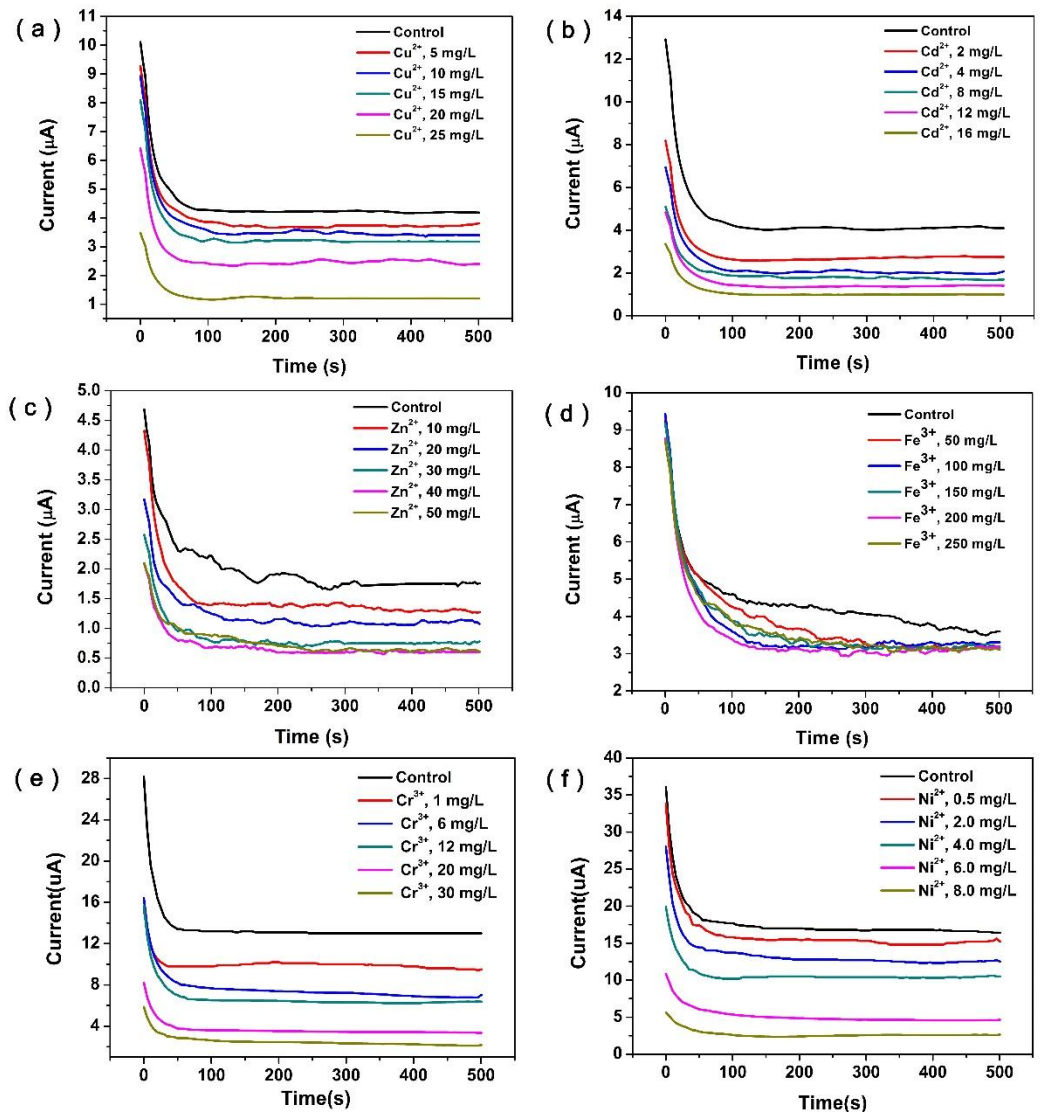


Fig. 4. Responses of *E. coli* to Cu^{2+}, Cd^{2+}, Zn^{2+}, Fe^{3+}, Cr^{3+} and Ni^{2+} by the isolation method.

Table1. Comparisons of the IC_{50} values of Cu^{2+}, Cd^{2+}, Zn^{2+} and Ni^{2+}obtained by the isolation method with that from the reported mediated electrochemical microbial biosensors.

Biotoxicity assays	Mediator	Under-test chemicals, IC_{50} (mg/L)				Ref.
		Cu^{2+}	Cd^{2+}	Zn^{2+}	Ni^{2+}	
E. coli; Isolation method	ferricyanide	21.3	3.7	26.7	4.4	This study
E. coli; MICREDOX ®	ferricyanide	>150	-	-	-	(Liu et al. 2009)
E. coli; FM-TOX	ferricyanide	3.71	7.8	7.5	1.9	(Catterall et al. 2010)
Activated Sludge; FM-TOX	ferricyanide	-	13.42	1.189	-	(Ma et al. 2016)
E. coli; CellSense biosensor	*p*-benzoquinone	80	>80	>80	95	(Li et al. 2013)
Psychrobacter sp; ToxTell biosensor	*p*-benzoquinone	2.6	47.3	10.9	-	(Wang et al. 2013)
E. coli; BGSH biosensor	*p*-benzoquinone	44	79	-	-	(Li et al. 2014)

We have applied this isolation method to toxicity assessment of Cu^{2+}, Cd^{2+}, Zn^{2+}, Fe^{3+}, Ni^{2+} and Cr^{3+} and the results are shown in Fig. 4. The calculated IC_{50} values of Cu^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and Cr^{3+} were 21.3 mg/L (R^2=0.96), 3.7 mg/L (R^2=0.99), 26.7 mg/L (R^2=0.96), 4.4 mg/L (R^2=0.98) and 10.7 mg/L (R^2=0.95), respectively. The inhibition caused by Fe^{3+} of 25 mg/L is about 15%, but the inhibition effect was nearly the same with the further increase of concentration of Fe^{3+} upper to 250 mg/L (p value = 0.1358). According to the inhibition effect of these four heavy metal ions, the toxicity order from high to low was Cd^{2+}> Ni^{2+} > Cr^{3+} > Cu^{2+}> Zn^{2+}> Fe^{3+}. Table 1 exhibits the IC_{50} values of Cu^{2+}, Cd^{2+}, Zn^{2+} and Ni^{2+} obtained by the isolation method were comparable to that from the reported mediated electrochemical microbial biosensors.

Relative standard deviation (R.S.D) and detection limits (LODs) were shown some analytical characteristics of this method. The precision of the procedure was determined by R.S.D obtained for five replicate assays for each metal ion at the same

concentration. The R.S.D values were 3.51%, 2.11%, 5.26%, 23.39%, 4.34% and 4.29% for Cu^{2+}, Cd^{2+}, Zn^{2+}, Fe^{3+}, Ni^{2+} and Cr^{3+}, respectively. The LODs were calculated by the equation: LODs= $3\sigma_b/s$, where σ_b is the standard deviation of blank measures obtained from 11 blank samples without toxicants, s is the slope of fitted linearity at low concentration of the metal ions. The LODs values for Cu^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and Cr^{3+} were 0.46 mg/L, 0.26 mg/L, 0.45 mg/L, 0.17 mg/L and 0.53 mg/L, respectively.

5. Evaluating the toxicity of real water samples using new mediated biosensor constructed by an isolation method

In addition to assess toxicity of heavy metal ions (Cu^{2+}, Cd^{2+}, Zn^{2+}, Fe^{3+}), the isolation method is also applied to the toxicity evaluation of four real water samples: effluents from landfill, electroplating wastewater, wastewater from chemical laboratory and the tap water (Fig. S5). As shown in Fig. S5, the steady currents of the control group and group of effluents from landfill were nearly the same, indicating the daily-used tap water performed no toxicity (p value = 0.1272). While the steady currents of three left wastewater groups were lower than the control group, suggesting they were of toxicity to some extent. According to the steady current values of these four real water samples, the toxicity order of them from high to low was electroplating wastewater > effluents from landfill > wastewater from chemical laboratory > tap water. The successful toxicity evaluations of these four real water samples also suggested the proposed isolation method can be applied for the practical and complex samples.

6. Conclusions

In this study, for the first time, we have systemically investigated the three interference factors existing in the present mediated electrochemical microbial biosensors by taking heavy metal ions as examples: (1) the potential reactions between the under-test chemicals (heavy metal ions) and mediators; (2) the electrochemical sensitivity of mediators to pH; (3) the potential reactions between under-test chemicals (heavy metal ions) and buffers and the consequent pH changes.

We found that all these interference factors stemmed from the 'one-pot' design concept, in which under-test chemicals directly mixed with mediators or buffers. Therefore, a new mediated biosensor based on an isolation design concept was developed, which can avoid these three interference factors existing in the present "one-pot" mediated electrochemical microbial biosensor system, leading to higher stability and sensitivity. We believe the interference problem analysis and the proposed isolation method (solutions) can give new insight to toxicity assessment by the mediated electrochemical microbial biosensors.

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Highlights

- In this study, interference factors existing in conventional mediated electrochemical microbial biosensors are systematically investigated.
- An “isolation method” is introduced to resolve the interference factors existing at present mediators electrochemical biosensor.
- The as prepared biosensor proved to be sensitive and reliable in real wastewater sample detection.