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A novel integrated biosensor based on co-immobilizing the mediator and microorganism for water biotoxicity assay†

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A novel integrated biosensor for biotoxicity assay has been developed by co-immobilizing microorganisms and mediators within a novel redox hydrogel. The proposed redox hydrogel acts as an immobilizing matrix both for microorganism *E. coli* and redox mediator, which was prepared by grafting the benzoquinone (BQ) redox mediator with gelatin/silica hybrid hydrogel (GSH). This redox hydrogel was characterized by UV-Vis, CV and EIS. The feasibility of the novel integrated biosensor for biotoxicity assay was demonstrated by measuring the heavy metal ions Hg^{2+}, Cu^{2+} and Cd^{2+} polluted water as the model toxicants. The results showed that the integrated biosensor was able to evaluate the water biotoxicity and the corresponding 50% inhibiting concentrations (IC_{50}) are determined to be 21.2 μg mL^{-1}, 44 μg mL^{-1} and 79 μg mL^{-1}, respectively. This integrated biosensor could achieve real-time monitoring of water quality and evaluation of biotoxicity. Moreover it avoids the waste and contamination of mediators, and also simplifies the assay process.

1. Introduction

The rapid and reliable evaluation of water quality continues to be an important issue in water safety, since conventional chemical/physical methods have limitations related to the inability to evaluate the bioavailability of the pollutants or to foresee interactive effects of pollutants in complex water matrices. Therefore, there is considerable interest in developing biological methods to study toxicity and measuring the risk of life-threatening chemicals in the environment. The environment monitoring methodology based on a direct biotoxicity assessment has attracted increasing scientific interest, due to its capability to make the assessment of overall composite toxicity and a promising approach for early risk warning of acute water toxicity.

Typically, for a biotoxicity assessment, the sensing element is a living organism, such as algae,[1] luminescence bacteria,[2] plant tissues,[3] animal fish,[4] *etc.*, and the essential sensing scheme relies on monitoring certain physiological changes in this living organism under the effect of an environmental stimulus. However, many of these assessments are time-consuming and not suitable for real-time determination or on-line measurement. Thus, some schemes based on monitoring the respiration chain activity of microorganisms have attracted considerable attention.[5–7] The respiration chain activity and thereby the biotoxicity could be easily determined by electrochemically measuring the change in dissolved oxygen concentration. However, the lower and unstable concentration of dissolved oxygen has hindered the practical application of such assays.[8]

Consequently, recent studies which deal with the use of an artificial electron mediator instead of natural dissolved oxygen to accept the electrons in living organism respiration, *i.e.*, redox-mediated biotoxicity assays have been undertaken.[8–13] The mediator is reduced during its interaction with the microbial respiration chain and the reduced mediator is then quantified by an electrochemical method, such as amperometric detection.[14] Compared with O_2, the employment of redox mediators shows high sensitivity and stability for biotoxicity assays and it allows for higher microbial populations without a rapid depletion of the electron acceptor. This is the basic principle of the first-generation biotoxicity assay. However, it seems that the performance of these sensors is still not satisfactory for practical application. For example, all the mediators reported in the mediated biotoxicity assays were usually applied in the aqueous solution,[8–13] and their concentrations were kept at a high level, *i.e.*, 60 mmol L^{-1} (ref. 9) or 45 mmol L^{-1}.[10] The high mediator concentration not only causes the poisoning of the microorganism, but also leads to waste and second contamination. Moreover, adding the redox mediator into the solution also makes it inconvenient for on-line and rapid detection. To the best of our knowledge, the co-immobilization of redox-mediators and microorganisms in the same matrix film to modified electrodes for biotoxicity assays has not been reported.

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An ideal strategy for obtaining direct redox control is the application of modified electrodes, *i.e.*, redox mediator and sensing element can be simultaneously entrapped onto the electrode surfaces. Such modified electrodes can not only avoid addition of a large amount of mediator, but also facilitate a real-time and rapid assay. However, the immobilization of redox mediators is more difficult than that of biocomponents, because mediators are usually water-soluble, small molecules which can easily diffuse away from the electrode surface. Generally, immobilization of the mediators on the electrode can be achieved through different strategies, such as dispersion of the mediator in the bulk of a composite electrode or adsorption, covalent attachment or entrapment of the mediator on the electrode surface,[15] *etc.* Benzoquinone and some organic compounds, as redox mediators, due to their neutrality and lack of modifiable functional groups, are often physically absorbed on the electrode for enzyme biosensors[16,17] and therefore the leaching of the mediator from the matrix would potentially diminish the performances of the modified electrodes.

Furthermore, based on the above discussion, an appropriate immobilized matrix is also very important. An alternative approach is to employ a redox polymer hydrogel as the three-dimensional matrix for immobilization of microorganisms. The redox polymer contains the pendants of redox species and the backbone polymer. A soft, flexible, and hydrophilic redox gel would not only be beneficial for electron transfer, but also be good for ensuring homogeneous contacts between the microorganisms and mediators.[18] Besides, as an immobilized matrix, it is known that a single component has its own inherent obstacles, such as crackling, swelling and less compatibility, while organic–inorganic hybrid components can effectively eliminate the brittleness of pure inorganic materials and the swelling properties of some pure polymers or hydrogels.[19,20]

In this study, we present the elaboration of a functional organic–inorganic electroactive redox hydrogel (benzoquinone/gelatin/silica hydrogel, BGSH) by combining silica, gelatin and redox mediator benzoquinone (BQ). 3-Aminopropyltrimethoxysilane (APTMOS), one of the silane-coupling agents, was selected to cross-link gelatin to prepare the gelatin/silica hybrid hydrogel (GSH) and the redox mediator BQ was grafted onto the silica backbone by the amino group of GSH to prepare benzoquinone/gelatin/silica hydrogel (BGSH). The integrated microbial biosensor was fabricated by immobilizing *E. coli* within the BGSH on the glassy carbon (GC) electrode. Finally, the integrated microbial biosensor was characterized by CV and EIS, and also employed to monitor and evaluate the biotoxicity of polluted water of heavy metals. The biotoxicity responses and IC_{50} results of heavy metals such as Hg^{2+}, Cu^{2+} and Cd^{2+} are presented and discussed.

2. Experimental

2.1. Reagents and solutions

3-Aminopropyltrimethoxysilane (APTMOS) was purchased from Alfa Aesar. Other chemical reagents used were of analytical grade (Beijing Lanyi Chemical Products Co., Ltd., China) and all solutions were prepared using deionised water obtained from a Millipore Milli-Q purification system (M-Q water, >18 MΩ cm). The phosphate buffer (PBS) was prepared from sodium dihydrogen phosphate and disodium hydrogen phosphate. The supporting electrolyte was PBS of pH = 7.0, 0.1 M. The nutrients as a respiratory substrate were prepared by mixing sodium lactate, sodium succinate and glucose each at 10 mmol L^{-1} in PBS (pH = 7.0, 0.1 M). Benzoquinone was dissolved in PBS (pH = 7.0, 0.1 M). All toxicant samples were freshly prepared in Milli-Q water from high-concentration stock solutions.

The wastewater from our laboratory was chosen for the real sample analysis without any treatment. The amount of tested wastewater was calculated in accordance with the volume ratio of tested wastewater to respiratory solution (v/v). Wastewater with the amount from 0.2% to 0.7% (v/v) was measured with the prepared biosensors.

2.2. Biological material

E. coli (ATCC 25922) was obtained from the China General Microbiological Culture Collection Center and replanted regularly on nutrient agar plates to ensure its viability. A 50 mL solution of autoclaved broth (0.25 g NaCl, 0.15 g yeast extract, 0.5 g peptone) was inoculated with a colony of *E. coli* and aerobically incubated at 37 °C for 16 h on an orbital shaker. The cell paste was harvested by centrifugation at 5000 rpm for 10 min at room temperature, then washed twice in PBS and re-suspended in PBS. The concentration of cells was adjusted to the desired optical density at 600 nm (OD_{600}).

2.3. Apparatus

Cyclic voltammetric and chronoamperometric experiments were performed with a 263A potentiostat/galvanostat (Princeton, USA). A conventional three-electrode system was used with a bare GC electrode or a modified GC electrode as the working electrode, a Ag/AgCl electrode (saturated with KCl) as the reference electrode, and a platinum stick as the auxiliary electrode. Electrochemical impedance spectroscopy (EIS) was carried out with a FRD 100 frequency response detector (Princeton, USA) connected to the same potentiostat/galvanostat. The surface area of the working electrode was 0.08 cm^2. All measurements were carried out at room temperature. The experiment of OD_{600} was conducted by using a UV spectrophotometer (SECOMAM UVIKONXL).

2.4. Preparation of GSH and BGSH

The gelatin/silica hydrogel (GSH) was obtained by hydrolysis of APTMOS in gelatin solution. Gelatin solution (5 wt%) was prepared by dissolving gelatin in 10 mL PBS and stirred for 30 min at 37 °C. And then an appropriate amount of APTMOS as well as HCl was added to initiate the cross-linking reaction. The GSH mixture was stirred at 50 °C until a homogenous solution was obtained.

For the preparation of redox hydrogel (BGSH), 0.04 M benzoquinone solution was added into the GSH (1/5, v/v). The mixture was stirred gently at 40 °C for 6 h and then the wine hydrogel was obtained. The BGSH was freshly prepared daily before the fabrication of biosensors.

The solution of poly(3-aminopropyl)siloxane (PAPS) was prepared by hydrolysis of APTMOS mixed with 0.50 mol L^{-1} HCl (1/10, v/v). The PAPS solution was mixed with 0.04 M benzoquinone (1/4, v/v), and the mixture was stirred at 40 °C for 6 h. Then the solution was poured into a large amount of acetone to precipitate the PAPS–BQ product. The precipitates were collected by filtration, washed with acetone, and then dried at room temperature.

2.5. Biosensor preparation

Prior to electrode modification, a glassy carbon (GC) electrode was polished well with 1, 0.3, and 0.05 μm alumina powders respectively, rinsed thoroughly with deionised water between each polishing step, ultra-sonicated in 1 : 1 nitric acid, acetone and deionised water in succession, and then allowed to dry at room temperature.

The BGSH was mixed with *E. coli* suspension at a proportion of 1 : 1 (v/v). Then, 8 μL of the mixed solution was dispensed on the surface of the GC electrode and allowed to dry at room temperature. The microbial biosensor was then washed thoroughly with PBS to remove the loosely bound mediator. The microbial biosensor was freshly fabricated during experiments and stored at 4 °C.

2.6. Measurements

The biotoxicity assessments were conducted by chronoamperometry (0.4 V, *vs.* Ag/AgCl) under continuous and constant magnetic stirring (700 rpm). After about 600 seconds for stabilization, heavy metal ions were added into the stirring respiratory solution.

The inhibition ratio was used to evaluate the biotoxicity of added toxicants. It was calculated by eqn (1):

$$\text{inhibition } (\%) = 1 - i_2/i_1 \quad (1)$$

where i_1 stands for the currents before the addition of toxicants; i_2 stands for the currents of 1000 s after the addition of toxicants.

3. Results and discussion

3.1. Synthesis and characterization of the electroactive BGSH

As one kind of protein, gelatin obtained by partial degradation of collagen has gained more attention for its film-forming ability. But as a protein film, the gelatin film does not have ideal mechanical properties which limited its application as biomaterials. In order to increase the mechanical strength of the immobilized polymer matrix and further graft redox mediators, we synthesized an electroactive benzoquinone functionalized silica and gelatin hybrid hydrogel matrix to immobilize the microorganism, *E. coli*, and then to modify the GC electrode to prepare a microbial biosensor for the biotoxicity assay.

The redox hydrogel BGSH was synthesized by reacting BQ with gelatin/silica hydrogel (GSH) solution which was obtained by the hydrolysis of APTMOS in the gelatin solution. The amino group of BGSH coming from PAPS, which is a hydrolysis product of APTMOS (Scheme 1a), could react with BQ by 1,4-addition reaction to produce a substituted quinone,[21] *i.e.*, the amine group replaces the hydrogen of BQ and formed hydroquinone and substituted hydroquinone (Scheme 1b).

UV-Vis spectral studies were carried out to confirm the linkage of PAPS to benzoquinone. As shown in Fig. 1, before the interaction of BQ and GSH, the absorption of the GSH in the 200–600 nm range was negligible (Fig. 1a), and there is an obvious absorbance peak at 246 nm for BQ in BGSH (Fig. 1b). As the reaction proceeded for 6 hours, a noticeable variation of the UV-Vis spectra was observed with a new absorbance peak at 289 nm and 346 nm, respectively, and a decrease of peak at 246 nm (Fig. 1c). Similar results are shown in Fig. S1† for the interaction of pure PAPS with BQ (gelatin was not present). The absorbance at 289 nm represented hydroquinone[22] which disappeared after washing with acetone (Fig. S1d†), whereas the substituted hydroquinone was subsequently oxidized to form the substituted quinone which corresponded to the new absorbance at 346 nm, and it was still observed after washing with acetone, indicating that the quinone was indeed grafted in the polymeric chain of the GSH (PAPS). Such results were in accordance with related work which shows similar UV-Vis spectra.[23]

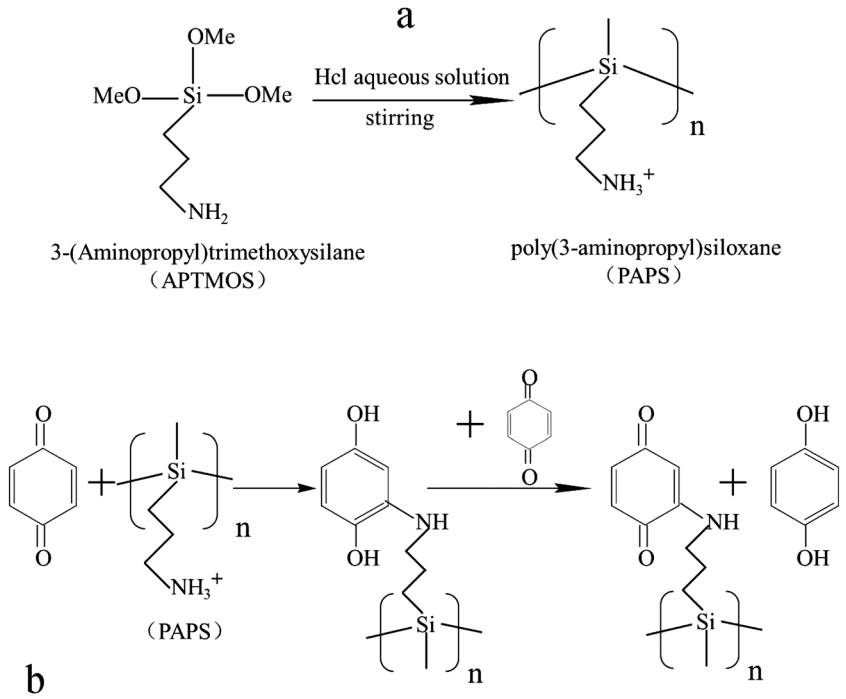
Based on the above results, it can be concluded that APTMOS, the inorganic precursor of GSH, not only cross-links the gelatin to reinforce the mechanical strength of the hybrid hydrogel, but also provides the amine group to graft the quinone, and therefore conferred the redox mediator to the organic–inorganic hybrid hydrogel.

3.2. Electrochemical characteristics of the BGSH modified GC electrode

Before immobilization of the sensing element *E. coli*, the immobilization of quinone groups as well as the ability to exchange the electrons between BGSH redox hydrogel and electrode surface was studied by cyclic voltammetry (CV) and EIS.

The CV performance of the BGSH modified GC electrode was investigated in a 0.1 M PBS (pH 7.0) solution at different scan rates. As shown in Fig. 2a, a couple of well-defined redox peaks due to quinone groups of BGSH were stable upon cycling, which implied a remarkable electrochemical cyclic stable species involved in the charge transfer process. ΔE_p is about 1.03 mV which does not fulfill the criteria of reversibility, indicating that the transduction of charge through hydrogel is not fast enough. It might be attributed to the non-conductive chain of hydrogel which could hinder the electron transfer. This behavior is also usually observed in the case of chemically modified quinone on ITO electrodes.[24] Furthermore, a linear relationship between the scan rate and peak currents was observed (the inset of Fig. 2a), confirming the surface controlled redox process (grafted quinone). Such an observation is in agreement with related studies.[25]

One of the main factors in determining the performance of the hydrogel modified electrode for bioelectronic application is the electron transfer efficiency. EIS is a useful tool for



Scheme 1 (a) Hydrolysis of APTMOS to PAPS; (b) reaction process of benzoquinone with PAPS to form substituted quinones.

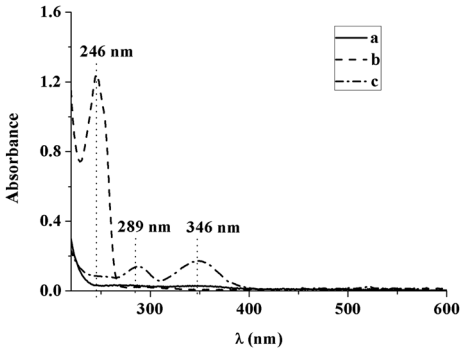


Fig. 1 UV-Vis spectra of GSH (a), BGSH (b) of 0 h, and BGSH (c) of 6 h.

characterizing the interface feature and electron transfer process of the modified electrode. The typical impedance spectrum includes a linear part at a lower frequency range corresponding to the diffusion limited process and a semicircular part at higher frequencies representing the electron transfer limited process. The diameter of the semicircle is equal to the electron transfer resistance (R_{et}) of the redox probe at the electrode interfaces. The EIS experiments were performed in a solution of 0.1 M KCl containing 5.0 mM $Fe(CN)_6^{3-}$ and 5.0 mM $Fe(CN)_6^{4-}$ at a applied potential of 0.2 V with the frequency ranging from 10^4 to 10^{-1} Hz and an alternating current voltage of 5 mV. The inset in Fig. 2b shows the most frequently used equivalent circuit for modeling the EIS experiments, Randles equivalence circuit model, which contains the electrolyte resistance (R_S) of the bulk solution in series with a double-layer capacitance (C_{dl}), electron-transfer resistance (R_{et}), and Warburg impedance (Z_W). As can be observed, significant differences of EIS results were observed upon the stepwise formation of the modified electrode. The R_{et} of a bare GC electrode was estimated to be 219 Ω. The deposition of the GSH film on the GC electrode caused a R_{et} of 446 Ω, which implied that the GSH film has restricted the electron transfer of the electrochemical probe toward the electrode surface. When BQ was grafted onto the GSH to form BGSH, the BGSH/GC electrode showed a R_{et} decrease compared with that of the no-BQ grafted GSH/GC electrode. R_{et} was calculated to be 91 Ω. This improved electron transfer property could be easily attributed to the introduction of BQ into the GSH. However, the electron transfer resistance largely increased to 503 Ω after *E. coli* was immobilized, suggesting a decrease in conductivity due to the incorporation of non-conductive biomacromolecules.

The above results indicated that BQ grafted onto the hydrogel well maintained the electrochemical activity, which ensured the application in the following redox-mediated assays.

Besides, during the experiment, we also observed that the combination of gelatin with silica can effectively overcome the swelling of the hydrogel matrix. Therefore, a soft, flexible, and hydrophilic redox hydrogel would not only be beneficial for electron transfer but also be good for ensuring homogeneous contacts.

3.3. Electrochemical behaviors of the *E. coli*/BGSH electrode

The BGSH was designed as a co-immobilizing matrix for microorganisms and redox mediators, and the results above also have shown that the BGSH could provide a favorable environment for grafted BQ with electron transfer. Therefore, the integrated microbial biosensor was further fabricated by immobilizing *E. coli* with the BGSH to modify the GC electrode.

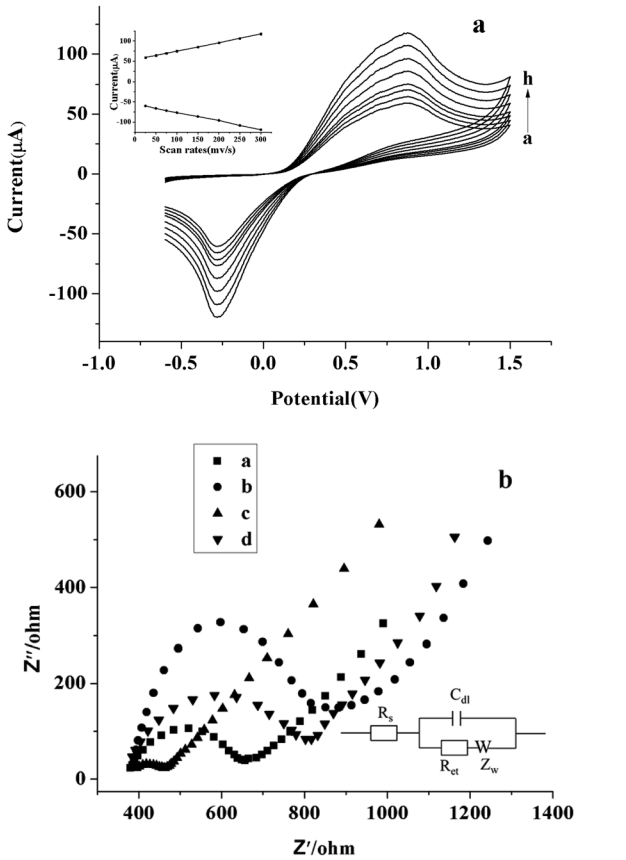


Fig. 2 (a) Typical cyclic voltammograms of the BGSH modified GC electrode at different scan rates (from a to h): 25, 50, 75, 100, 150, 200, 250, and 300 mV s^{-1} in the 0.1 M phosphate buffer (pH 7.0). The inset shows the peak current dependence on the scan rate, ν. (b) EIS for the (a) bare GC electrode, (b) GSH/GC electrode, (c) BGSH/GC electrode and (d) *E. coli*/BGSH/GC electrode in a solution of 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-}$ and 5 mM $Fe(CN)_6^{4-}$. Inset: the Randles equivalent circuit model for modified electrodes.

The CV behaviors of the modified GC electrode were investigated in a 0.1 M PBS (pH 7.0) solution in the absence and presence of the respiratory substrate in pH 7.0 PBS after 5 min of incubation at a scan rate of 50 mV s^{-1}. As shown in Fig. 3, in the absence of the respiratory substrate, the *E. coli* biosensor prepared just gave a pair of redox peaks (Fig. 3a) which was attributed to quinone groups in BGSH, while with the addition of the respiratory substrate into the solution, the anodic peak current increased noticeably and cathodic peak current decreased (Fig. 3b). This behavior is consistent with a very strong electro-catalytic effect. The reaction mechanism was proposed and listed below:

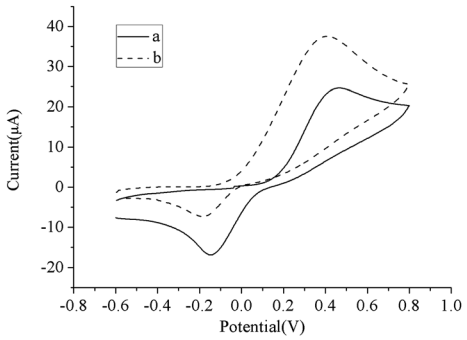


Fig. 3 Cyclic voltammograms of the *E. coli*/BGSH/GC electrode in the absence (a) and in the presence (b) of the respiratory substrate in pH 7.0 PBS after 5 min of incubation at a scan rate of 50 mV s^{-1}.

Through the metabolic reaction, *E. coli* oxidized the respiratory substrate and electrons derived from this process were then transferred to reduce the mediator existing in the redox hydrogel. The reduced BGSH was electrochemically re-oxidized on the electrode and thus leads to the increased anodic peak current. These indicated that the changes of peak currents just originated from the metabolic activity of *E. coli* and thus further demonstrated that the BGSH redox hydrogel can act as a mediator and immobilized matrix for *E. coli* in the application of the mediated microbial process.

Besides, gelatin is a kind of nontoxic and biocompatibility material for the immobilization of microbial cells and is capable of maintaining the maximum microbial activity. Mixing gelatin with silica not only enhances the flexibility, but also effectively decreases the swelling of the matrix.

3.4. Performances of the microbial biosensor prepared

The next step in this study was to apply the microbial biosensor prepared above to some toxic heavy metals. As described above, the redox hydrogel BGSH is reduced by immobilized *E. coli* through metabolic reaction and then re-oxidized on the surface of the GC electrode. Thus, the measurement of *E. coli* respiratory activity and the perturbation by pollutants can be detected as a change in the magnitude of the current. For this propose, the biotoxicity evaluation described in the Experimental section was carried out with three heavy metal ions. As expected, the addition of metal ions caused the decline of the current within about 50 seconds because of their detrimental effect to metabolic activity of *E. coli* (Fig. 4b–d). Comparatively, as shown in Fig. 4a, no obvious current change could be observed when no metal ions were introduced to the solution.

$$\text{respiratory substrate} + \text{redox hydrogel (Ox)} \xrightarrow{E.\ coli} \text{metabolite} + \text{redox hydrogel (Red)}$$
$$\text{redox hydrogel (Red)} \xrightarrow{\text{electrode}} \text{redox hydrogel (Ox)} + e^-$$

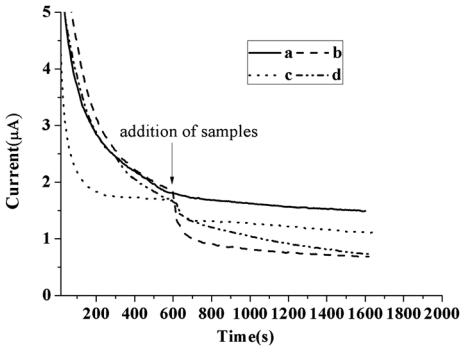


Fig. 4 The response curves of the addition of polluted water samples, (a) without toxicants, (b) with 30 μg mL^{-1} Hg^{2+}, (c) 20 μg mL^{-1} Cu^{2+}, and (d) 80 μg mL^{-1} Cd^{2+}, respectively.

The inhibitory curve obtained by measuring the inhibitions of microbial biosensors exposed to three heavy metal ions with different concentrations is presented in Fig. 5. As shown in Fig. 5a–c, their inhibitions all increase rapidly with increased concentrations, and moreover, different inhibition effects were observed upon different heavy metal ions, accordingly, the values of IC_{50} were estimated to be 21.2 μg mL^{-1}, 44 μg mL^{-1} and 79 μg mL^{-1}, respectively; the inhibitory effects of the three heavy metals can be ranked in a decreasing order of Hg^{2+} > Cu^{2+} > Cd^{2+}, which is in accordance with the results reported by conventional colony counting methods.[26]

The IC_{50} results of the present biosensor are compared with those of other biotoxicity assays, which are listed in Table 1. The IC_{50} values of the present biosensor are comparable to those of other redox-mediated assays[10,11] and other different methods, for example nitrification method and respirometer method.[26] The sensitivity of the present study was lower than that of Wang *et al.*'s report.[13] However, it should be noticed that, compared with the methods which disperse mediator,[13] or disperse both the microorganism and the mediator[10,11] into the solution, our co-immobilization strategy of microorganism (*E. coli*) and mediator may cause microorganism and mediator to work under unfavorable conditions, such as less free mobility of BQ, lower concentration, *etc.* Thus, the improvement of the co-immobilized system is needed for further investigation. Nevertheless, more importantly, the present integrated biosensor exhibits no requirement for adding any chemicals during the detection, and another advantage is that it can provide a constant monitoring of the microbial activity, which makes it possible for the real-time monitoring of water quality and early warning of emergent pollution.

3.5. Analysis of wastewater samples

In environmental pollution, a wide variety of chemicals simultaneously exist in water. The laboratory wastewater was measured as the real sample which was a mixture of heavy metals. The wastewater with the amount of 0.2% to 0.7% (v/v) was measured with the prepared biosensor. As shown in Fig. 5d, the integrated biosensor was sensitive to the real laboratory wastewater and 0.52% (v/v) could cause 50% inhibition.

3.6. Reproducibility and stability of the integrated microbial biosensor

The reproducibility of the integrated microbial biosensors was evaluated by measuring the responses of five replicates to 15 μg mL^{-1} Hg^{2+}. As shown in Fig. S2(a),† the prepared biosensors

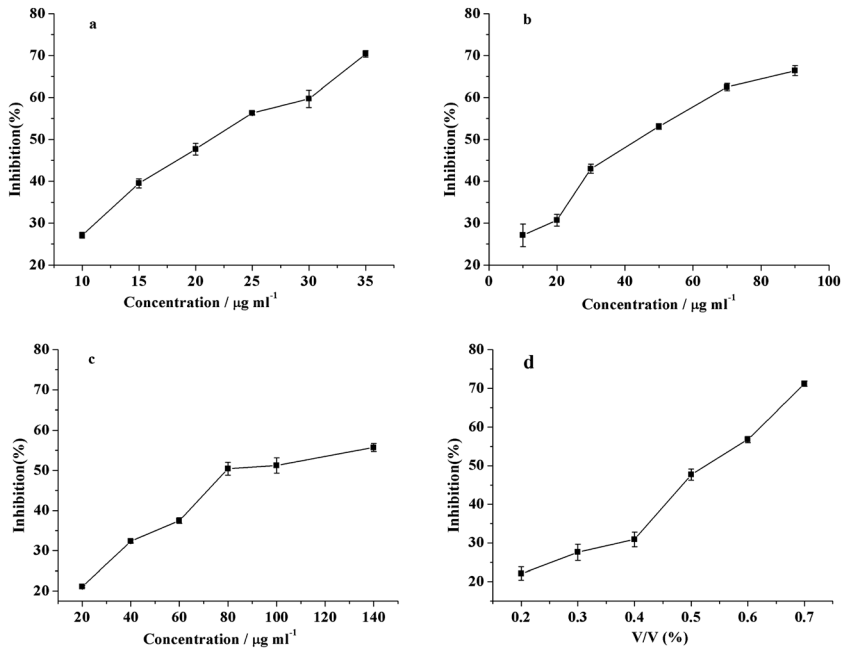


Fig. 5 Inhibitory curves of Hg^{2+} (a), Cu^{2+} (b) and Cd^{2+} (c) at different concentrations, and (d) the biotoxicity assay of real wastewater. Data points represent the average of three replicates.

Table 1 Comparison of IC_{50} values obtained from the present study and other biotoxicity assays reported

Toxicants, IC_{50} ($\mu g\ mL^{-1}$)				
Hg	Cu	Cd	Reference	Biotoxicity assays
21.2	44	79	Present study	*E. coli*; biosensor
39.36	—	93.95	(ref. 11)	*E. coli*; amperometry
40	60 (IC_{20})	—	(ref. 10)	*E. coli*; amperometry
—	41.5	33.1	(ref. 27)	Nitrifying bacteria; nitrification method
—	40	61.1	(ref. 27)	Activated sludge; respirometer method
0.8	2.6	47.3	(ref. 13)	*Psychrobacter* sp. biosensor

exhibited a good and reproducible performance with a relative standard deviation (RSD) of 2.33%.

When the stability was concerned, the integrated microbial biosensors were kept at 4 °C. The responses to 15 $\mu g\ mL^{-1}$ Hg^{2+} were measured every two days and the results showed a relative standard deviation (RSD) of 3.71% for ten days as shown in Fig. S2(b).†

4. Conclusions

In this report, we designed and synthesized a novel organic–inorganic hybrid redox hydrogel by co-immobilization of *E. coli* and mediator BQ into a hydrogel. An integrated microbial biosensor was developed based on this hybrid redox hydrogel. The present results show that the prepared biosensor was effective for determination of heavy metal toxicity in water. The corresponding 50% inhibiting concentrations (IC_{50}) for biotoxicity assay are determined to be 21.2 $\mu g\ mL^{-1}$ for Hg^{2+}, 44 $\mu g\ mL^{-1}$ for Cu^{2+} and 79 $\mu g\ mL^{-1}$ for Cd^{2+}. The proposed integrated microbial biosensor was novel, rapid and convenient for biotoxicity assay of chemicals and environmental water monitoring.

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