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Colorimetric and Electrochemical Dual-Signal Method for Water Toxicity Detection Based on *Escherichia coli* and *p*-Benzoquinone

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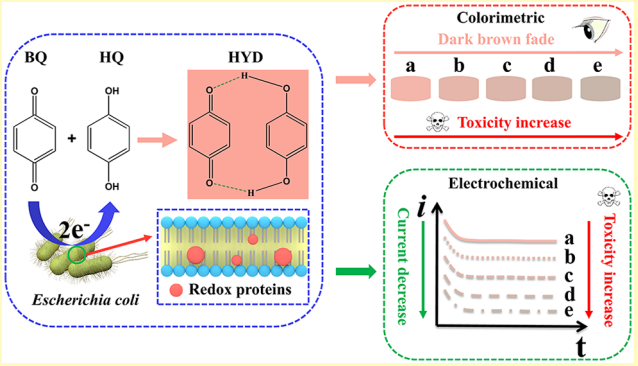
ABSTRACT: The development of simple and rapid toxicity detection methods has important practical significance. In this work, a dual-signal method with colorimetric and electrochemical properties for water toxicity detection was proposed for the first time based on a rapid color reaction between *Escherichia coli* (*E. coli*) and *p*-benzoquinone (BQ). Here, *E. coli* was used as a biocatalyst and BQ was used as a mediator. An IC_{50} value of 0.75 mg L^{-1} for Cu^{2+} was obtained using a two-step electrochemical detection method. Strikingly, toxicity could also be estimated visually by the naked eye, and the minimum detection limit was 3.2 mg L^{-1} for Cu^{2+}. The dual-signal toxicity detection method extends the function of BQ, and the result is more reliable than the traditional single-signal method. This simple and rapid toxicity detection method shows certain application prospects.



KEYWORDS: *biosensor, colorimetric, toxicity, Escherichia coli (E. coli), p-benzoquinone (BQ)*

Water pollution is becoming increasingly serious with the rapid development of industry and agriculture. Various types of pollutants, such as heavy metal ions and pesticides, with high toxicity in water seriously threaten the safety of human beings. Environmental factors are complicated, and the synergistic, antagonistic, and combined effects of toxic pollutants and other substances, as well as changes in environmental conditions, affect toxicity.[1] At this time, a biological toxicity monitoring method is needed to monitor whether water is polluted and to determine the degree of pollution. Algae,[2] zebrafish,[3] daphnia,[4] and microorganisms[5] have been used as indicators for water toxicity detection. Among them, microbial toxicity detection has attracted much attention due to advantages such as easy operation, fast response, high sensitivity, and low cost.[6] Some experimental data show that there is a certain correlation and comparability between IC_{50} values obtained from cell-based experiments and animal experiments.[7] Many kinds of water toxicity biosensors based on luminescent bacteria have been developed in recent decades.[8] However, it is necessary to add 3% NaCl to the test solution,[9] which causes a change in the composition of the test solution and introduces detection errors.[10] In addition, spectral detection is affected owing to the chromaticity and turbidity of the test solution.[11] In recent years, the study of water toxicity detection using electrochemical methods that can be applied in turbid media has received extensive attention.[11,12] However, this approach is not suitable for on-site emergency disposal of pollution accidents due to the need for large instruments and cumbersome testing procedures required for the water toxicity detection methods described above.

Colorimetric sensors have become a research hotspot in recent years because of their simple and visible detection procedure.[13] Yong et al.[14] reported that 3,5-dichlorophenol (3,5-DCP) at 10 mg L^{-1} could be estimated visually by the naked eye through a mediator-free method based on genetically modified *Pseudomonas aeruginosa*. Pujol-Vila et al.[15] constructed a colorimetric biosensor by fixing $K_3[Fe(CN)_6]$ and *Escherichia coli* (*E. coli*) on filter paper with glutaraldehyde. The toxicity was determined according to the chromaticity analysis of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ obtained by microbial reduction. *p*-Benzoquinone (BQ) is well known as a lipophilic redox mediator, which can be used in electrochemical biosensors for determining toxicity,[16] glucose levels,[17] and substrate-oxidizing activity.[18] The use of BQ-mediated recognition elements in toxicity biosensor systems has become common. Wang et al.[19] successfully developed a simple, rapid, and reliable amperometric-mediated biosensor, which adopted BQ as a mediator, to determine the toxicity of heavy metal pollutants to the activated sludge

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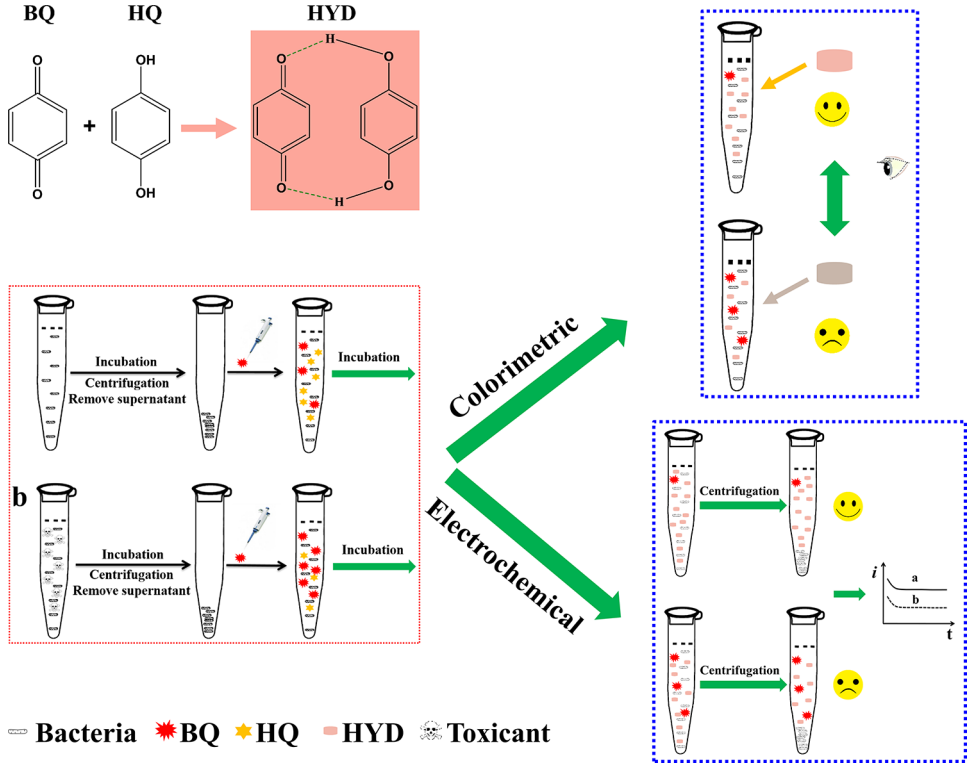
Scheme 1. Schematic Illustration of Colorimetric and Electrochemical Dual-Signal Method for Toxicity Detection



environment. Zhi et al.[16] proved that BQ was more efficient for biotoxicity assay than $K_3[Fe(CN)_6]$ in a simple microbial BQ-mediated biosensor fabricated by immobilizing *E. coli* on a glassy carbon electrode with gelatin solutions. However, these methods only used a single electrochemical method to assay water toxicity. Sun et al.[20] reported a new BQ-mediated *E. coli* detection system through colorimetric and electrochemical analytic detection assays, in which *E. coli* can be identified from four other types of bacteria including *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mutans*, and *Salmonella pullorum*. Here, the detection object was the pathogenic bacteria *E. coli*, and the electrochemical detection was the reduction current of unreacted BQ.

In this work, a novel BQ-mediated biosensor with a dual-signal involving both colorimetric detection and electrochemical detection was reported. The principle of electrochemical detection is based on the difference of the oxidation current of hydroquinone (HQ) obtained by reducing BQ with bacteria in water toxicity detection. The color reaction between *E. coli* and BQ is not specific as compared with other bacteria. It is suitable for the development of rapid toxicity detection sensors based on the rapid color reaction of *E. coli* and BQ. The feasibility of the proposed biosensor was examined by determining the toxicity of Cu^{2+}. The sensitivity of water toxicity detection was improved with the two-step electrochemical method. Colorimetric toxicity detection with visual analysis by the naked eye does not rely on any complex instruments. Dual-signal water toxicity detection makes the results more reliable.

EXPERIMENTAL SECTION

Chemicals and Reagents. BQ was acquired from Sinopharm Chemical Reagent Co., Ltd. NaOH, $Na_2HPO_4{\cdot}12H_2O$, and KH_2PO_4 were purchased from Beijing Chemical Plant. The yeast extract was purchased from Oxoid Co. Ltd. (U.K.). Peptone was purchased from Beijing Aoboxing Biotech Co., Ltd., and glycerol was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. Lysogeny broth (LB, 10% peptone, 5% yeast extract, and 10% sodium chloride) was adjusted to the desired pH = 7.4 with 1 M NaOH and then sterilized in a vertical pressure steam sterilizer (YXQ-LS-70A, Shanghai Boxun Medical Biological Instrument Co., Ltd.) for 20 min at 121 °C. BQ as a mediator was freshly prepared, wrapped with an aluminum foil, and kept at 4 °C in a refrigerator for further use. All toxicants at the desired concentration were freshly prepared by diluting the high-concentration stock solution (1000 mg L^{-1} Cu^{2+}). Stock solutions were freshly prepared or kept at 4 °C. A phosphate-buffered solution (PBS, 200 mM) was prepared as follows: 42.98 g of $Na_2HPO_4{\cdot}12H_2O$ and 10.89 g of KH_2PO_4 per liter were mixed. Unless otherwise stated, all chemicals were used as received without further purification, and all solutions were prepared using deionized water (>18.25 MΩ/cm) purified with a Millipore Milli-Q purification system (Millipore).

Instruments. Electrochemical measurements including cyclic voltammetry (CV) and chronoamperometry were performed with an electrochemical workstation (CHI 832B, Shanghai Chen Hua Instrument Co., Ltd.). Cells were cultured in an oscillator (ZHWY-200B, Shanghai Zhicheng Analytical Instrument Manufacturing Co. Ltd.). Cells and culture solutions were centrifuged with a centrifuge (5804 R, Eppendorf Co. Ltd.). The optical density (OD_{600}) of the mixed microbe suspension (*E. coli*) was obtained using a Cary 500 UV−vis−NIR spectrometer (Varian) at 600 nm.

Microbial Culture. *E. coli* (DH 5α) was obtained from the China General Microbiological Culture Collection Center (CGMCC) and maintained in LB containing 30% glycerol at −80 °C to ensure viability. Here, *cells* were cultured aerobically on an orbital shaker (200 rpm) in LB for 16 h at 37 °C. Then, the cells were harvested by centrifugation at 5000 rpm for 5 min at room temperature and washed twice with a NaCl solution. The remaining bacterial pellets were resuspended in a NaCl solution. The concentration of the *E. coli* suspension was assayed at 600 nm. Several other bacteria, mixed bacteria, *Bacillus subtilis* (*B. subtilis*), BODseed (Cole-Parmer E-05466-00), and *Shewanella oneidensis* MR-1 (MR-1) were cultured according to previous reports.[21] Then, they were washed and

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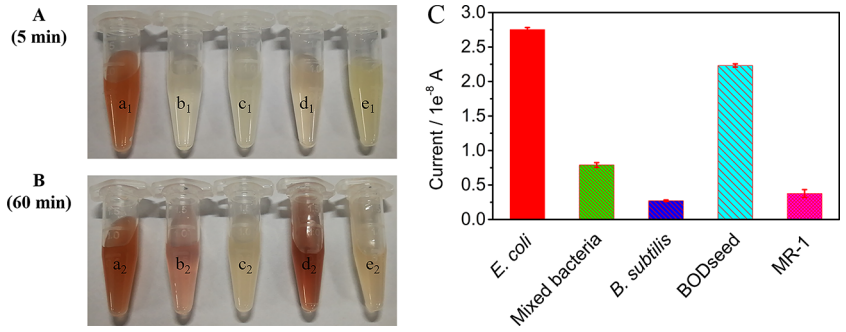

Figure 1. Colorimetric response of different bacteria to BQ for (A) 5 min and (B) 60 min at room temperature. (C) Histogram of the oxidation current after the reaction of different bacteria with BQ for 60 min at room temperature. (a) *E. coli*, (b) mixed bacteria, (c) *B. subtilis*, (d) BODseed, and (e) MR-1.

resuspended in PBS according to similar preparation procedures of *E. coli*.

Principle of the Toxicity Detection. The basic principle of toxicity detection is based on changes in the respiration chain activity of *E. coli* induced by toxicants. A schematic illustration of the toxicity detection is shown in Scheme 1. We know that the reaction product of the remaining unreacted BQ and HQ reduced by microorganisms from BQ was dark brown quinhydrone (HYD). More HYD was generated in the control sample in the absence of toxicants. However, the respiratory and metabolic activities of microorganisms were inhibited in the presence of toxicants, which resulted in less HQ production than that of the control sample. Accordingly, the amount of HYD was reduced, and the color of the toxic sample was lighter. Moreover, the color of the samples became lighter as the concentration of toxic substances gradually increased. The toxicity results showed that the lowest concentration (LC) could be visually estimated by the naked eye.

As shown in Figure S1, quantitative toxicity test results can be obtained by electrochemical methods. The oxidation current decreased gradually with increasing concentrations of toxic substances. The inhibition ratio is adopted to evaluate water toxicity and calculated according to eq 1

$$\text{inhibition}\ (\%) = 100\% \times (i_{con} - i_{tox})/i_{con} \quad (1)$$

here i_{con} and i_{tox} represent the oxidation currents obtained in the absence and the presence of toxicants, respectively.

Procedure of Toxicity Detection. The toxicity detection procedure for a sample containing heavy metal ions was performed using a two-step method according to a previous report.[22] In the first step, 0.9 mL of the cell suspension at the desired concentration and 0.1 mL of a toxic solution or deionized water were thoroughly mixed and incubated at 37 °C. Then, to completely remove heavy metal ions, the mixed solutions were centrifuged at 10 000 rpm for 10 min and washed twice with a NaCl solution. In the second step, 1 mL of BQ prepared with PBS was added to the washed *E. coli*. After the mixed solution was uniformly mixed by vibration, we could see that the colors of samples immediately changed to dark brown, and the colors of samples were basically stable after ∼5 min. Then, after an appropriate response time, the toxicity could be semiquantified by the color differences between the control sample and the toxic sample. Samples used for electrochemical testing were incubated again for 50 min at 37 °C. Then, the mixed solutions were centrifuged at 10 000 rpm for 10 min at room temperature, and the supernatant solutions obtained were electrochemically detected. The concentrations of HQ in the supernatant solutions were determined by chronoamperometry (0.3 V versus Ag/AgCl). The biosensing system was equipped by dipping a conventional three-electrode system into an electrochemical cell (1.5 mL standard centrifuge tube). The three-electrode system consisted of an as-prepared Pt microelectrode array (MEA)[21d] as a working electrode, a Pt wire electrode (CHI 115, Shanghai Chen Hua Instrument Co., Ltd.) as a counter electrode, and an Ag/AgCl electrode (CHI 111, 3 M KCl, Shanghai Chen Hua Instrument Co., Ltd.) as a reference electrode. The surface of MEA was polished with 0.05 μm γ-alumina slurry and subsequently sonicated twice in deionized water for 2 min. Unless otherwise stated, all potentials given here were versus the Ag/AgCl reference electrode. All electrochemical and colorimetric measurements were performed at room temperature.

RESULTS AND DISCUSSION

Color Reactions between Bacteria and BQ. As shown in Figure 1, *E. coli* and four other bacteria with the same optical density values of OD_{600} = 3 were mixed with BQ (2 mM). As shown in Figure 1A, after 5 min of the reaction, it was surprising that only the color of the sample containing *E. coli* changed to dark brown, while the colors of the samples containing other microorganisms did not change to dark brown. However, the color of the samples of mixed bacteria and BODseed gradually changed to dark brown over time. As shown in Figure 1B, after 60 min of the reaction, almost all of the samples were more or less dark brown. The color of the mixed bacterial sample changed to light dark brown, and the color of the BODseed sample was close to that of the *E. coli* sample. The color changes of the *B. subtilis* sample and the MR-1 sample were very small. The color of the sample of mixed bacteria changed to slightly dark brown, which may be related to the sample containing a small amount of *E. coli*. However, BODseed is a mixture of seven species of bacteria (*P. aeruginosa*, *Klebsiella oxytoca*, *Serratia liquefaciens*, *Yersinia enterocolitica*, *Citrobacter amalonaticus*, *Enterbacter sakazakii*, and *Enterobacter cloace*). Its color change after 60 min was almost the same as that of the *E. coli* sample, except that the color change was not as rapid as that of the *E. coli* sample. Therefore, the abovementioned results indicate that it is not a specific reaction between *E. coli* and BQ. The color change is attributed to the following reasons. The bacteria react with BQ to produce HQ, and then HQ reacts with the remaining unreacted BQ to produce HYD. The mechanism for the production of HQ from a chemical reaction between bacteria and BQ is shown in Figure S2. The microbial respiratory chain involves complex multistep redox reactions among various proteins in the cells. As shown in Figure S2A, BQ would be reduced to HQ by the redox center of FADH/FAD in bacteria under the influence of an external environment. As shown in Figure S2B, the reaction of BQ being reduced to HQ by bacteria can be carried out because the redox potential of BQ/HQ is higher than that of FADH/FAD.[22] The sample immediately turned dark brown when *E. coli* was mixed with BQ, and the dark brown color was basically stable after ∼5 min, indicating that the reaction was very fast. Therefore, there

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is a possibility of developing a rapid biosensor for water toxicity detection based on the reaction of *E. coli* and BQ.

The histogram of the oxidation current after the reaction of different bacteria with BQ for 60 min is shown in Figure 1C. It can be clearly seen from the figure that higher currents were obtained for the *E. coli* sample, the BODseed sample, and the mixed bacteria sample, which indicated that more HQ was generated. The largest amount of dark brown HYD, the largest oxidation current, and the fastest speed were obtained based on the reaction between *E. coli* and BQ. The oxidation currents after the reaction of different bacteria with BQ for 60 min are shown in Table S1. These results are attributed to the high activity and degradation ability of *E. coli*. Therefore, the combination of *E. coli* and BQ was the best choice to construct the colorimetric and electrochemical dual-signal biosensor.

Optimization of *E. coli* Concentration. In our previous research, the influence of the concentration of microorganisms and BQ on the sensitivity of water toxicity detection was optimized.[23] The sensitivity decreased with increasing BQ concentration in the range of 0.625−10.0 mM. Therefore, BQ (0.625 mM) was chosen as an optimal mediator concentration in this study. The sensitivity increased with increasing microbial concentration (OD_{600} = 2.4, 4.8, and 9.6). As shown in Figure S3, the reaction time of *E. coli* with BQ was optimized with an electrochemical method. The current rapidly increased in the range of 1−50 min, and then the current basically remained stable. Therefore, 50 min was chosen as the optimal reaction time with an electrochemical reaction between *E. coli* and BQ. As shown in Figure 2, with

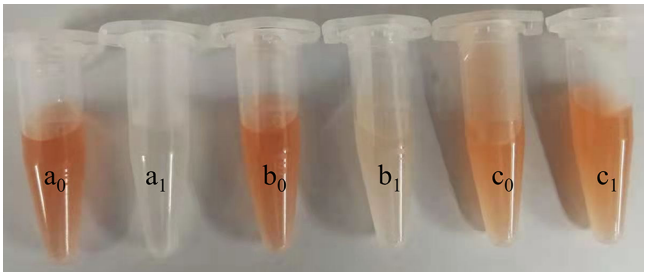

Figure 2. Optimization of the *E. coli* concentration with 0.625 mM BQ; the image was taken after BQ was added and mixed at room temperature. a_0, b_0, and c_0 are the control samples with OD_{600} = 2.4, 4.8, and 9.6 *E. coli*, respectively. a_1, b_1, and c_1 are the samples with 5 mg L^{-1} Cu^{2+} added to the corresponding concentration *E. coli*, respectively.

the concentration of microorganisms decreased in the range of OD_{600} = 2.4−9.6, the color of the control samples gradually darkened, while the color of the toxicity samples gradually lightened. Therefore, the largest color difference was observed between the control sample and the toxic sample for OD_{600} = 2.4 *E. coli*, and the smallest was the color difference for OD_{600} = 9.6 *E. coli*. It is a common understanding that greater color contrast between the control sample and the toxicity sample in colorimetric toxicity detection methods indicates greater sensitivity. Therefore, the colorimetric detection time is the time when the color difference between the control sample and the toxicity sample is the largest. It is not advisable to develop dual-signal water toxicity detection with OD_{600} = 9.6 *E. coli*, even though the electrochemical method has the highest sensitivity in the microbial concentration. As a compromise, OD_{600} = 4.8 *E. coli* was chosen as the microbial concentration to carry out the following experiments, in which higher sensitivity was obtained with electrochemical and colorimetric methods. A comparison of the color change of the solution with different reaction times between *E. coli* and BQ is shown in Figure S4. It was clearly observed that the color reaction between *E. coli* and BQ quickly reached a stable state. Dark brown could persist for at least 50 min at room temperature, which was sufficient for toxicity assessment.

Optimization of NaCl Concentration. The precipitate was generated, resulting in false-positive results because PBS reacts with heavy metal ions in one-step electrochemical toxicity detection of wastewater containing heavy metal ions. We also found that the color difference of wastewater containing different concentrations of heavy metal ions was small in one-step electrochemical toxicity detection. A possible reason was that the reaction of heavy metal ions with PBS reduced its concentration and resulted in less damage to microorganisms. Figure S5 shows the precipitate generated by the reaction of Cu^{2+} at different concentrations with 200 mM PBS. Therefore, a two-step method was adopted for toxicity detection in this work. In the first step, NaCl was used to replace PBS, which avoided the reaction between phosphate and heavy metal ions. The optimization of the NaCl concentration is shown in Figure 3. The current increased

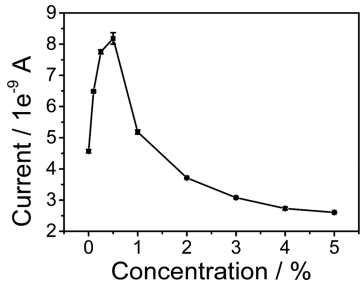

Figure 3. Optimization of the NaCl concentration; BQ = 0.625 mM, OD_{600} = 4.8 *E. coli*, incubation for 1 h at 37 °C.

with increasing NaCl concentration in the range of 0−0.5%. The current gradually decreased in the range of 0.5−5%. Therefore, a 0.5% NaCl solution was chosen as the final concentration for subsequent experiments. Because *E. coli* is a freshwater bacterium, its activity can be reduced or cells can undergo death due to osmotic pressure in a NaCl solution that is too high or too low. Current values for samples of different NaCl concentrations containing 0.625 mM BQ and OD_{600} = 4.8 *E. coli* incubated for 1 h at 37 °C are shown in Table S2.

Validation of Feasibility. To verify the feasibility of the constructed biosensor for toxicity detection, the changes in bacterial respiratory activity were measured. The experiment was carried out according to a two-step method. It should be noted that the final concentrations of cells, BQ, and Cu^{2+} were OD_{600} = 4.8, 0.625 mM, and 5 mg L^{-1}, respectively. Sample b and sample c were incubated for 1 h in a 37 °C shaker at 220 rpm. As shown in Figure 4, the detection current of each sample quickly reached a stable state within 10 s. Anodic currents were not observed for sample a (Figure 4A, curve a). The color of sample a in Figure 4B also did not change, which indicates that BQ could not be oxidized at a voltage of 0.3 V. As shown in Figure 4A, curve b, a large anode current appeared after BQ and bacteria were mixed and incubated for 1 h and 50 min. As shown in Figure 4A, curve c, the mixture containing BQ and cells was also incubated in the presence of 5 mg L^{-1}

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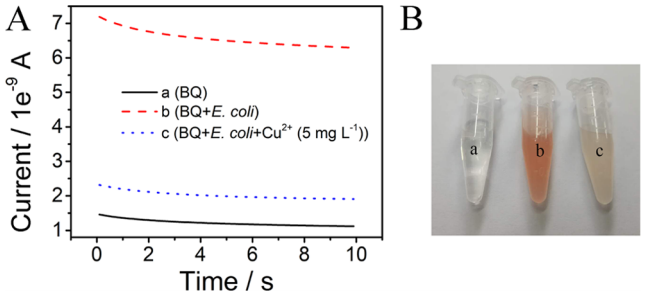

Figure 4. (A) $I-t$ curves recording electrochemical signals for the samples of (a) BQ, (b) *E. coli* and BQ (culture for 1 h and 50 min), and (c) BQ, *E. coli*, and Cu^{2+} (5 mg L^{-1}, culture for 1 h and 50 min); (B) image corresponding to the samples in (A).

Cu^{2+}. Although the anodic current still increased relative to sample a, the current was lower than that of sample b. The color changes of each sample in Figure 4B show the consistency of the current changes in Figure 4A. It should be noted here that the image was taken after BQ was added and mixed at room temperature. In particular, the color difference was great between sample b and sample c in Figure 4B, and the color of sample c became significantly lighter after Cu^{2+} was added. These results demonstrated that the BQ-mediated respiratory activity of cells can be inhibited by Cu^{2+}. The mean inhibition ratio of 5 mg L^{-1} Cu^{2+} was 47.53% (50.44, 44.92, and 47.24%) calculated according to the current values. Therefore, the constructed biosensor can be used to detect water toxicity with a colorimetric and electrochemical dual-signal method.

Stability of the Biosensor. As shown in Figure 5, the stability of MEA, electrochemical, and colorimetric biosensors of water toxicity detection was investigated, respectively. In general, all quinone compounds are strongly adsorbed on the electrode surface because of the lack of an electronic structure and hydrogen bonding. Therefore, to reduce the error, the MEA was passivated by scanning at least 200 CVs in a BQ solution before testing. Figure 5A shows that the current changes were very small after 100 CV cycles scanned using MEA as a working electrode in a 2 mM BQ (0.1 M KCl as a supporting electrolyte) solution, which indicated that the performance of MEA was stable. As shown in Figure 5B, the detection current values had a little difference (maximum 6.94%) across 20 control samples containing 0.625 mM BQ and OD_{600} = 4.8 *E. coli* cultured for 50 min at 37 °C, which indicated that good stability of the electrochemical detection signal was achieved for the biosensor. As shown in Figure 5C, the colors of the corresponding 20 samples in Figure 5B after BQ was added and mixed at room temperature were consistent, which further proved that the stability of the colorimetric biosensor was reliable. The oxidation current and the relative values of 20 parallel samples containing 0.625 mM BQ and OD_{600} = 4.8 *E. coli* cultured for 50 min at 37 °C are shown in Table S3.

Toxicity Assay. For the electrochemical toxicity assay, current−time curves were used to quantitatively measure the HQ obtained by reducing BQ with bacteria. On the basis of the abovementioned optimization conditions, the curve of the toxicity inhibition ratio of heavy metal ions of Cu^{2+} is shown in Figure 6A. IC_{50} = 0.75 mg L^{-1} of Cu^{2+} was obtained using OD_{600} = 4.8 *E. coli* as biological subjects in this work. The IC_{50} value of 0.75 mg L^{-1} was less than 0.95 mg L^{-1} obtained with a one-step method using OD_{600} = 9.6 *E. coli* as biological subjects in a previous report.[23] The abovementioned results indicated that the sensitivity of the biosensor with a two-step method was improved. This was attributed to the following reasons. The interference of heavy metal ions of Cu^{2+} with PBS and BQ was eliminated in the two-step method. It is valuable that the two-step method derived a new colorimetric toxicity detection method in water. As shown in Figure 6B, the color gradually became lighter with increasing Cu^{2+} concentration, which can be clearly distinguished by the naked eye. The image was taken after BQ was added and mixed at room temperature. The sample of 3.2 mg L^{-1} Cu^{2+} can be easily identified as having an obvious color difference from the control sample by naked eye colorimetric detection. Figure S6 shows an image of the water toxicity samples assayed by the one-step method. As seen from the figure, the sample colors darkened as Cu^{2+} concentrations increased, which may be resulting from the mixed color caused by the blue precipitate generated by the reaction between Cu^{2+} and PBS and the dark brown HYD. Therefore, the toxic effect of Cu^{2+} could not be truly reflected by the one-step method. Toxicity inhibition ratios of samples of different Cu^{2+} concentrations containing 0.625 mM BQ and OD_{600} = 4.8 *E. coli* incubated for 1 h and 50 min at 37 °C are shown in Table S4.

Table 1 shows the comparison of the IC_{50} values of Cu^{2+} obtained from this study and other reported mediated biotoxicity assays. It is generally accepted that a lower IC_{50} value corresponds to higher detection sensitivity for toxicity detection. It is obvious from Table 1 that the electrochemical detection sensitivity of the suspension method is higher than that of the fixed microbial method, which is attributed to the effective mass transfer efficiency of the suspension microbial method between microorganisms and the mediator. The two-step method is more sensitive than the one-step method, and

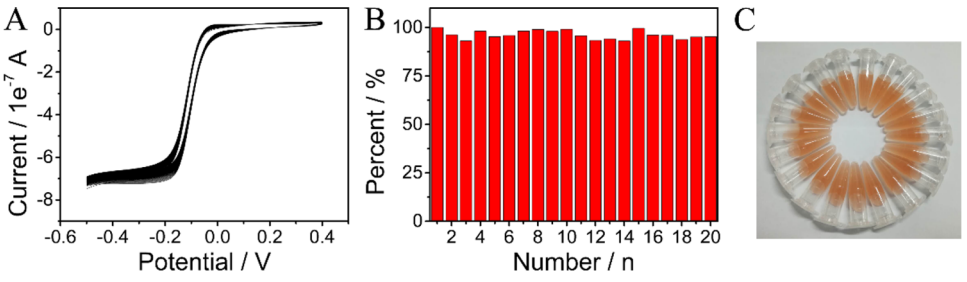

Figure 5. Stability of the biosensor of (A) 100 cycle CV curves of a sample containing 2 mM BQ in 0.1 M KCl at a scan rate of 50 mV/s; (B) oxidation current relative values of 20 parallel samples containing 0.625 mM BQ and OD_{600} = 4.8 *E. coli* cultured for 50 min at 37 °C; and (C) image corresponding to the samples in (B).

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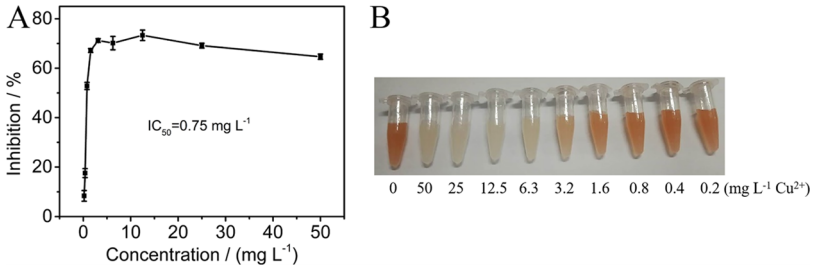

Figure 6. (A) Toxicity inhibition ratio of Cu^{2+} ions; BQ = 0.625 mM, OD_{600} = 4.8 *E. coli*, incubation for 1 h and 50 min at 37 °C; (B) image of colorimetric toxicity detection of Cu^{2+} ions.

Table 1. Comparison of the IC_{50} Values of Cu^{2+} Obtained from This Study and Other Reported Mediated Biotoxicity Assays

toxicity assays	mediator	microorganism	IC_{50} (mg L^{-1})	response time[a]	reference
amperometry	BQ	*E. coli*	0.75	60 min	this study
FM-TOX	$K_3[Fe(CN)_6]$	*E. coli*	21.3	120 min	22
amperometry	BQ	*E. coli*	0.95	60 min	23
BGSH biosensor	BQ	*E. coli*	44	real-time	25
ToxTell biosensor	BQ	Psychrobacter sp.	2.6	real-time	12b
CellSense biosensor	BQ	*E. coli*	80	real-time	16
FM-TOX	$K_3[Fe(CN)_6]$	*E. coli*	3.71	60 min	6a

[a]Time needed for interaction between the toxic sample and the microorganism.

the reason has been described in this article. The addition of organics to the samples also reduces the detection sensitivity, which has also been reported.[24] Furthermore, it can be seen from Video S1 that colorimetric detection results were obtained after ~1 min, which suggests that the method is very suitable for rapid toxicity detection. Therefore, the two-step method broadens the options for water toxicity detection, for which colorimetric detection is more suitable.

CONCLUSIONS

In this study, a novel dual-signal biosensor for water toxicity detection was developed based on the color reaction between *E. coli* and BQ. The toxicity of Cu^{2+} was successfully assayed by electrochemical and colorimetric methods. Cu^{2+} has high toxicity and a calculated IC_{50} value of 0.75 mg L^{-1} based on an electrochemical assay, which is more sensitive than the one-step toxicity detection reported previously. Although the colorimetric method was not as sensitive as the electrochemical method, the toxicity could be visually estimated by the naked eye with a toxicant concentration as low as 3.2 mg L^{-1}. The advantage of the combined approach is that the results are more reliable, making them more applicable to a wider range of scenarios. In conclusion, the dual-signal water toxicity biosensor using the color reaction between BQ and *E. coli* provides a simple and rapid detection method and shows a potential application value in the field of toxicity detection in water. In future, we plan to develop portable colorimetric toxicity equipment based on smartphone apps utilizing this technology.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.1c00651.

The schematic illustration of toxicity detection by the electrochemical method, the mechanism for the production of HQ from a chemical reaction between bacteria and BQ, optimization of culture time, comparison of the color change of the solution with different reaction times between bacteria and BQ, the image of precipitate generated by the reaction of Cu^{2+} and 200 mM PBS, the image of the samples of water toxicity assayed by the one-step method, the oxidation current after the reaction of different bacteria with BQ, current values for different NaCl concentrations, the oxidation current and the relative values of 20 parallel samples, and toxicity inhibition ratios of samples of different Cu^{2+} concentrations, dynamic comparison of color changes between the control sample and the test sample with 5 mg L^{-1} Cu^{2+} (PDF)

Video S1 (MP4)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

BQ, *p*-benzoquinone; HQ, hydroquinone; HYD, quinhydrone; CV, cyclic voltammogram; MEA, microelectrode array; LB, lysogeny broth; 3,5-DCP, 3,5-dichlorophenol; PBS, phosphate-buffered solution; LC, lowest concentration; IC_{50}, half-maximal inhibitory concentration

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