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A portable instrument for monitoring acute water toxicity based on mediated electrochemical biosensor: Design, testing and evaluation

Yajie Yang [a, b], Yanran Liu [a], Yafei Chen [a, b], Yu Wang [c], Peng Shao [c], Runze Liu [a], Guanyue Gao [a, b, **], Jinfang Zhi [a, b, *]

[a] Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, 100190, PR China
[b] University of Chinese Academy of Sciences, Beijing, 100049, PR China
[c] Beijing Center for Physical and Chemical Analysis, Beijing, 100089, PR China

HIGHLIGHTS

- A water-quality early warning instrument is developed for evaluating acute water toxicity.
- The instrument is based on *E. coli* integrated electrochemical biosensor.
- The results of multiple wastewater samples by the instrument show a good correlation with ISO Luminescent bacterial test.
- The instrument has a more excellent performance for colored water samples than ISO Luminescent bacterial test.

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ABSTRACT

A water quality early-warning instrument for evaluating acute water toxicity based on the electrochemical biosensor, Model ETOX18-01, was developed and manufactured with the features of low current detection (0.1 nA), precise thermostatic control, self-cleaning as well as remote data transmission. A sensitive integrated microbial electrode, made up of a glass carbon electrode that was modified by an active biofilm consisting of *Escherichia coli*, thionine, carbon nanodots and chitosan, has been fabricated as the biosensor. To validate the performance, multiple real water samples and artificial water samples were tested by Model ETOX18-01, and compared with ISO standardized luminescent bacterial test simultaneously. The correlation between the Model ETOX18-01 and luminescent bacterial test for these water samples showed good determination coefficient ($R^2 = 0.9827$). In addition, Model ETOX18-01 is more sensitive to colored metal ionic samples. With its characteristics of high sensitivity, excellent repeatability and easy operation, the instrument Model ETOX18-01 provides a promising tool for large-scale water environmental assessment, and has a potential application in evaluating the water quality and early risk warning.

1. Introduction

The life of living organism and human being is closely related to water environment (Rogers, 2006; Li et al., 2018). Quality criteria for aquatic environment is primarily based on bioassays or toxicity tests. Acute toxicity tests are the first step in determining acceptable levels of given chemical substances for a particular species. Measurements of acute water toxicity have been developed for many years with various principles by using organisms such as fishes, shrimps, and other aquatic animals as bioreceptors (Radix et al., 2000; Wang et al., 2013; Pujol-Vila et al., 2015; Turemis et al., 2018). But these methods are usually time-consuming and need sophisticated procedures to operate. So, methods based on microorganisms, especially bacteria, have been widely used and adopted as a supplement to predict water toxicity to humans and ecosystems (Ribo and Kaiser, 1987; Campo et al., 2007; Eltzov et al.,

* Corresponding author. Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, 100190, PR China.
** Corresponding author. University of Chinese Academy of Sciences, Beijing, 100049, PR China.
E-mail addresses: gaoguanyue@mail.ipc.ac.cn (G. Gao), zhi-mail@mail.ipc.ac.cn (J. Zhi).

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2015; Yu et al., 2017; Cui et al., 2018; Hassan et al., 2019). Among all the commercially available instruments, the most attractive mechanism is monitoring the metabolic activity of bioluminescent marine bacteria, such as *Aliivibrio fischeri* and *Photobacterium phosphoreum* (Zheng and Jialing, 1995; Parvez et al., 2006; Elad et al., 2011; Han-Chang et al., 2013). However, the drawback of bioluminescent marine bacteria is that they are not suitable for colored and high turbidity samples, which would cause false positive results. Furthermore, these bioluminescent marine bacteria need hyperosmotic and high salinity environment, which results in the low solubility of organic pollutants and thus limits the application of luminescent bacteria test.

In recent decades, amperometric microbial biosensors have been extensively exploited for environmental application (Mulchandani et al., 2001; Priti et al., 2006; Ben-Yoav et al., 2011; Wang et al., 2013; Ghanavati et al., 2014; Yong et al., 2015). The respiration processes of bacteria can be effectively monitored by electroanalytical techniques with redox mediators (Pasco et al., 2001; Tizzard et al., 2004; Morris et al., 2005; Ma et al., 2016). The mechanism is that the redox mediators can seize the electrons from respiratory chain of bacteria and be reduced, then it can be re-oxidized on the surface of electrode under certain potential and generate current signal. The activity of microorganisms will be affected by toxicants, leading to current intensity change. Therefore, the toxicity of pollution can be evaluated by obtained current intensity. CellSense, a mature commercial biosensor device, which measures bacterial respiratory activity, has already been used to detect toxic industrial compounds in environmental matrices (Evans et al., 1998; Farré et al., 2001, 2002; Farré and Barceló, 2003; Wang et al., 2008).

In present work, we developed a new electrochemical biotoxicity early-warning instrument, Model ETOX18-01, which was designed to be a sensitive, economical and compact monitor for water quality measurement. This Model ETOX18-01 instrument possesses the ability of low current detection to 0.1 nA, precise thermostatic control, self-cleaning as well as remote data transmission. As core part of the instrument, a high sensitivity integrated bacterial electrode was established by modified glass carbon electrode (GCE) with an active biofilm consisting of *Escherichia coli* (*E. coli*) and electron mediator thionine (TH). The mediator was absorbed by electrostatic interaction firstly, then immobilized into biocompatible composite of chitosan (CHI) and carbon nanodots (CNDs). Moreover, the properties of this integrated electrochemical biosensor were systematically studied and explored, including parameter optimization, stability testing, lifetime preservation. To validate the instrument performance, multiple real and artificial water samples were collected and tested by Model ETOX18-01 and ISO standardized luminescent bacterial test simultaneously. The results show that Model ETOX18-01 instrument is effective in water quality evaluation and early warning, which can be applied to measure the acute toxicity of a broad range of substances, including ground surface water, industrial wastewater, soil leachate and laboratory wastewater, etc.

2. Materials and methods

2.1. Chemicals

Escherichia coli (ATCC25922) was cultivated by China General Microbiological Culture Collection Center. *Photobacterium phosphoreum* 502 was purchased from Huaju Technology Company, Shenzhen, China. Thionine acetate was purchased from Alfa Aesa. Beef extract and peptone were purchased from Anbo Biotech Company, Beijing. All other reagents were provided by Beijing Lanyi Chemical Products Co. Ltd., China. All chemicals used were analytical grade without further purification. Ultrapure water ($R > 18.0$ MΩ cm) was obtained from Milli-Q water system. The samples of rivers, lakes and reservoirs were collected from Beixiao River, Ba River, Chaoyang Park Lake, Tuanjie River, Shisanling reservoirs and Chongqing River in Beijing, China, respectively. The other real water samples were provided by Beijing Center for Physical & Chemical Analysis, China. The artificial wastewater samples were prepared in our laboratory.

CHI flakes were dissolved in 0.1% (v/v) acetic acid solution to prepare chitosan solution (0.2%, w/v). The respiration substrate (RS) solution used during the experiment consisted of sodium lactate, glucose and sodium succinate with 0.85% sodium chloride solution.

CNDs, a kind of discrete and quasi-spherical nanoparticles, possess the merits of good conductivity, biocompatibility and cheapness, which can increase the conductivity of biofilms (Li et al., 2012; Zhao et al., 2015). They were synthesized as described in our previous work (Fang et al., 2016).

The concentration of *E. coli* was characterized by optical density (OD_{600}), which was obtained by measuring the absorbance at wavelength of 600 nm using UV−Vis spectrophotometer (SECOMAM UVIKONXL). The biofilm on the surface of electrode was characterized by scanning electron microscopy (SEM, HITACHI S-4800) with an operating voltage of 5 kV. The electrochemical impedance test was measured by electrochemical impedance spectrometer (Princeton FRD 100). The luminescent bacterial test (ISO standard method) were done by Hamamatsu BHP9514.

2.2. Construction of instrument model ETOX18-01

A water quality early-warning instrument based on the electrochemical biosensor, Model ETOX18-01, was developed. Fig. 1a shows a simplified system diagram of instrument appearance and components, and Fig. 1b is blocks diagram of instrument systems. As shown in Fig. 1a, the shape of the instrument is a rectangular parallelepiped and the specification is 40 (L) × 45 (W) × 65 (H) cm. The reaction cell is placed at the bottom of the device which is designed as double-layered glass container to provide a constant temperature surrounding by circulating water in the interlayer. The outside of the reaction cell is a cylinder (Φ 50 × 60 mm) and inside is a frustum of a cone (19 (R) × 5 (r) × 60 (H) mm).

The instrument is organized by three-electrode electrochemical measuring part, control part and display part. Three-electrode electrochemical measuring system includes a Pt wire as counter electrode, an Ag/AgCl (KCl sat') electrode as reference electrode, an integrated microbial electrode (IME) fabricated by a bioactive film modified onto the glassy carbon electrode (GCE, d = 5 mm) as working electrode, and electrolyte solution to form circuit for measuring the current signal. Control part is constituted by the automatic agitator, automatic temperature control, self-cleaning, signals collection, and automatic analysis. Automatic agitator is realized by the magnetic stirring coil underneath the reaction tank. Once the energized coil generates a magnetic field, the stirring bar is rotated and produces the stable mixture in the reaction vessel, where the solution can be mixed sufficiently. Automatic temperature control, which is designed to retain the temperature of the reaction cell for further improving the accuracy of the test results, is composed of a circulating water bath heating and temperature sensor whose precision can reach to 0.1 °C. A self-cleaning system is responsible to discharge the experimental liquid and clean the reaction kettle. It is equipped with three kinds of leaner to wipe off different kinds of tested samples and satisfy the cleaning requirements. Data acquisition and analysis are fully automated according to signals collection and processing unit. Specially, a micro-current detecting system is developed which allows an accurate

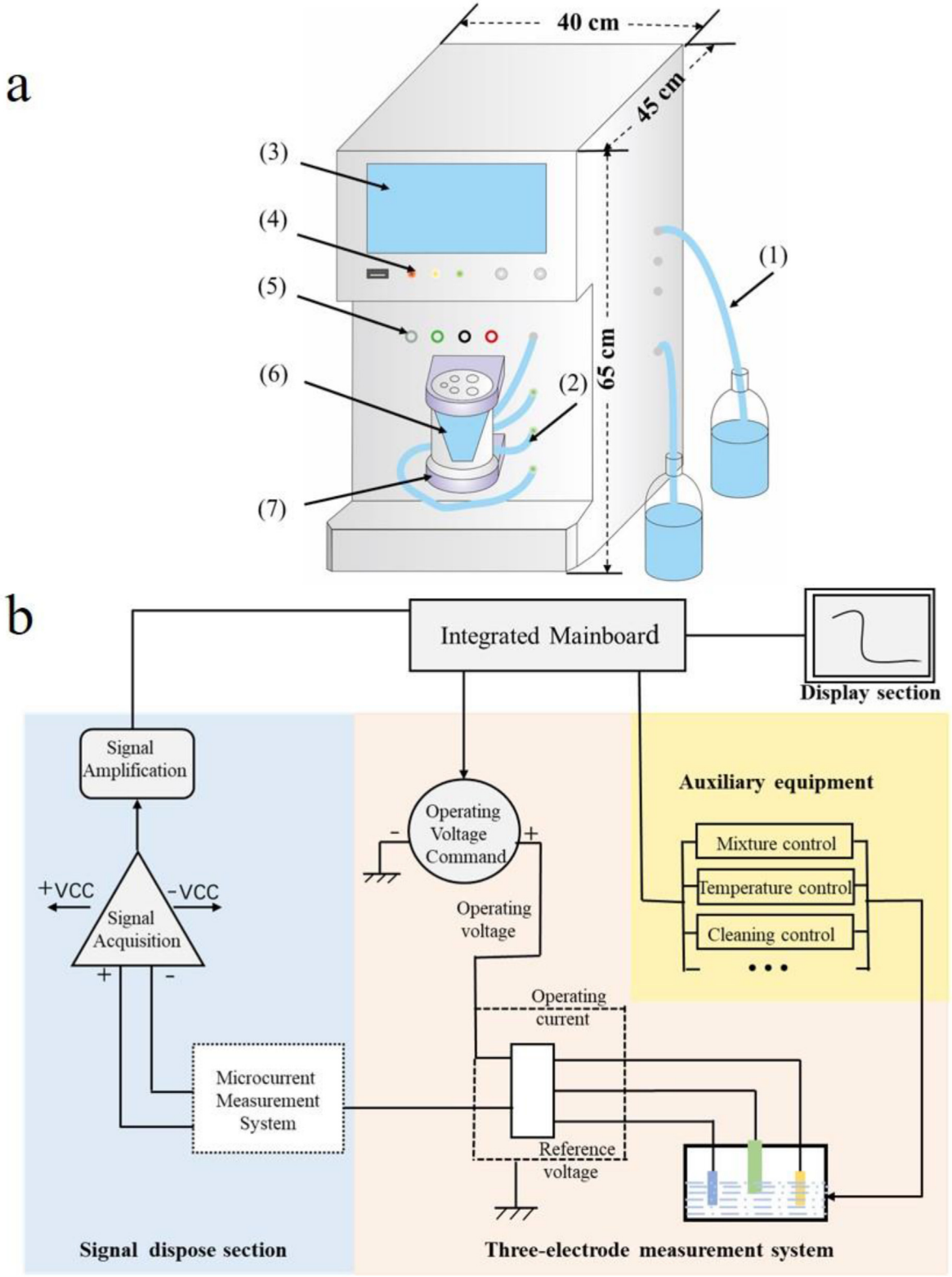

Fig. 1. (a) Diagram of instrument appearance and components, including: (1) self-cleaning; (2) automatic temperature control; (3) LCD display; (4) button control; (5) electrode wire interface; (6) reaction cell; (7) automatic agitator. (b) Block diagram of the Model ETOX18-01.

and reproducible determination of current changes. Display part consists of an LCD screen, a control panel and a video switcher. LCD touch screen is inset on the top of the detector and USB interface is equipped on the bottom of LCD screen to satisfy the data transferring demand.

The operation of Model ETOX18-01 is convenient. Firstly, the command is sent by integrated mainboard which inputs a voltage to the three-electrode electrochemical testing system and controls

the external reaction parameters, such as temperature, stirring speed. Then the three-electrode system outputs the current signal which is detected by micro-current detecting system, and this electrochemical signal is collected and amplified. Finally, signal is fed back to the integrated mainboard then become visual output display.

2.3. Preparation of integrated microbial electrode

The IME modified on the surface of GCE was made up of bioactive film, which was fabricated by *E. coli,* TH, CHI and CNDs. *E. coli* was used as a test organism and TH acted as redox mediator which can grasp electrons from the respiratory chain of *E. coli* and be reduced. Then reduced TH was oxidized on the surface of electrode at a suitable oxidation potential and produced current signal. The preparing scheme of IME is shown in Fig. 2. Firstly, *E. coli* was cultured aerobically at 37 °C for 16 h in a 180 rmp rotary shaker. The mixture was then centrifuged and washed twice with 0.85% (w/v) sodium chloride solution. UV–Vis spectrophotometer at 600 nm was used to adjust the concentration of *E. coli* suspension.

5 mL suspension was uniformly mixed with 20 mL TH solution (0.3 mM) and kept for 15 min. In neutral pH solution, the surface charge of *E. coli* is negative owing to its isoelectric point is around 5.6 (Sherbet and Lakshmi, 1973). TH contains sulfide bond which is commonly shown positive charge (Fang et al., 2016). Hence, TH can be absorbed on the surface of *E. coli* by electrostatic interaction. Then the mixture was centrifuged to remove the redundant TH. Finally, *E. coli*@TH was obtained and further diluted to 1 mL by 0.85% sodium chloride solution. Comparing mixed directly, *E. coli*@TH connected by electrostatic interaction will accelerate the electron transfer rate between TH and *E. coli*.

CNDs (0.2 mg L^{-1}), CHI solution and *E. coli*@TH were mixed adequately in the volume ratio of 1:2:2, then CHI–CNDs-*E. coli*@TH solution was dropped on the GCE surface to prepare CHI–CNDs-*E. coli*@TH/GCE (Fig. S1). The GCE electrode prepared was dried at room temperature and stored at 4 °C.

2.4. Acute water toxicity data acquisition and analysis

Before the detection, power-on and then set up reacted temperature, stirring speed and reacted period. After the preparatory work was ready, the RS solution (7 mL) as electrolyte was added into the reaction tank and the IME was inserted to the electrolyte. Then the start button was pressed and current was recorded. When the current was stable, the tested samples were added and the button was pressed again to start reaction countdown. The microorganisms, which were modified onto the IME, were used as toxicity bioreceptors. The respiration signal was converted into current signal by the redox mediator TH, and relationship between current and time was recorded by chronoamperometry (0.3 V *vs.* Ag/AgCl). The inhibition rate was calculated through the following Eq. (1) to obtain the toxicity of the samples:

$$\text{Inhibition ratio } (\%) = (1 - i_1/i_0) \times 100\% \quad (1)$$

where i_0 is the steady current before the samples were added, i_1 is the steady current after the samples were added or the current after the samples were added for 900 s if the current was unable to reach steady. The IC_{50} was obtained from the concentration–inhibition linear curve. Each toxicity assessment was repeated three times. The reliability of results was examined by Kolmogorov-Smirnov normality test and one-way ANOVA to analyze their normal distribution and significant difference (Tables S1, S2, S3 and S5).

2.5. Procedure of luminescent bacterial test

The freeze-dried power of *Photobacterium phosphoreum* 502 was stored at −20 °C and the bacterial suspensions used for toxicity assay should be freshly prepared before using. In preparation of bacterial suspensions, freeze-dried bacteria were evenly dispersed in resuscitation fluid (3% sodium chloride solution) and activated for 15 min at room temperature. Then 50 μL activated bacteria were added in a mixture of 100 μL osmoregulation fluid (30% sodium chloride solution) and 900 μL toxicant solution. The control group contained 50 μL activated bacteria, 100 μL osmoregulation fluid and 900 μL deionized water was also made. Luminescence was measured after 15 min reaction with either toxicant or deionized water. A commercial instrument, Hamamatsu BHP9514, was used for luminescence measurements at room temperature. The inhibition rate was calculated through the following Eq. (2):

$$\text{Inhibition ratio } (\%) = (1 - L_1/L_0) \times 100\% \quad (2)$$

where L_0 is luminescence of cells mixed with deionized water, and L_1 is the luminescence of cells mixed with sample. Each toxicity assessment was repeated three times.

3. Results and discussion

3.1. Characterization of the IME by EIS

An electrochemical impedance spectroscopy (EIS) was used to evaluate whether each component was immobilized on the GCE successfully. The IME was fabricated as mentioned in section 2.3. As illustrated in Fig. 3, the Nyquist plot of GCE appeared as a

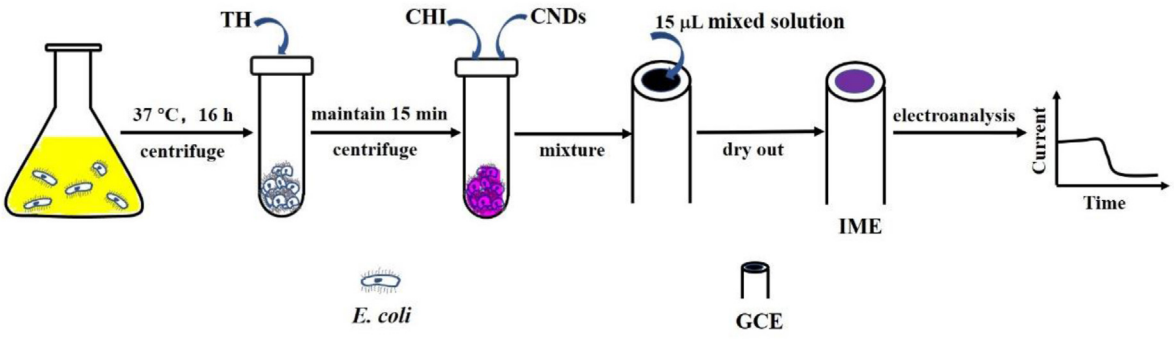

Fig. 2. The preparing scheme of IME and flow chart of electrochemical biosensor based on the IME.

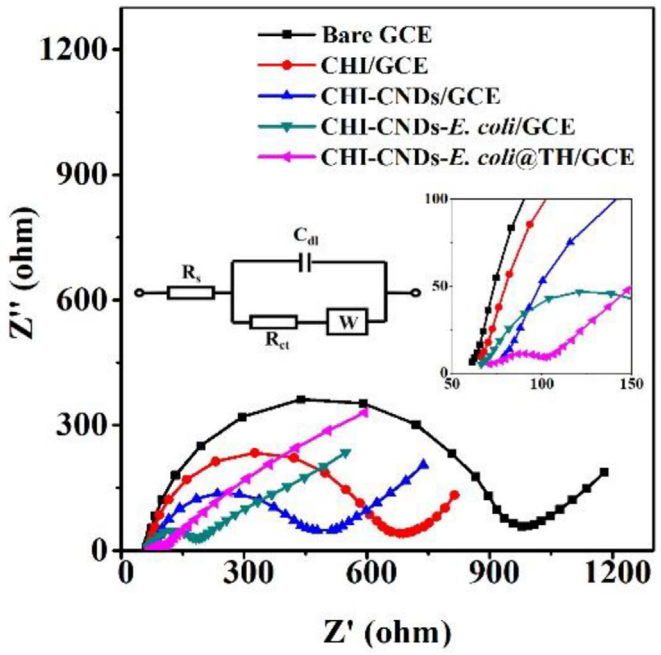

Fig. 3. Nyquist plots and equivalent circuit of GCE modified with different materials.

characteristic semicircle in high-frequency range and a slop in low-frequency range. And the corresponding equivalent electrical circuit also be given, where R_s is the internal resistance, C_{dl} is the double layer capacitance, R_{ct} is charge transfer resistance, and W is Warburg impedance. The semicircle in the Nyquist plot corresponded to R_{ct}, which was controlled by electrode reaction kinetics. Compared to the bare GCE, a significant decrease of charge transfer resistance was observed when CHI, CHI-CNDs, *E. coli* and TH were added. The charge transfer resistance displayed a gradual change indicating that the active components were successful modified on the surface of GCE. Meanwhile, it should be noted that CHI—CNDs-*E. coli*@TH/GCE only had 45 Ω charge transfer resistance, which was attributed to the shorten electron transfer distance between electroactive centers and GCE realized by wrapping the *E. coli* and TH together. Thus, the IME prepared exhibits an excellent electron transfer ability.

3.2. Optimization conditions of IME

As *E. coli* is signal recognition component for the biosensor, its concentration has a crucial influence on the sensitivity of sensors. In present system, OD_{600} between 1.5 and 3.5 were selected to study the sensitivity of the sensors and obtain the optimal experimental conditions. The Zn^{2+} was chosen as target pollutant in toxicity evaluation. The results were shown in Fig. 4a and Table S1 (P value < 0.05). As can be seen from Fig. 4a, the inhibition rate showed an increasing trend when the OD_{600} values varied from 1.5 to 2.5, and then a decrease was observed when the OD_{600} values were increased from 2.5 to 3.5. This phenomenon suggested that the detection sensitivity of biosensor for Zn^{2+} was in optimal condition when $OD_{600} = 2.5$. Therefore, $OD_{600} = 2.5$ was selected as the optimum concentration of *E. coli* in the subsequent detections.

For the microbial sensors, it is also significant to optimize the volume of CHI-CNDs-*E. coli*@TH solution dropped on the surface of GCE, because it can affect the thickness of active biofilm and change the detection sensitivity. The non-conductive CHI biofilm is more likely to split off and cause the imprecision of current signal when the biofilm is too thick. Otherwise the current signal will be low and hard to be detected if the CHI mixed solution volume is insufficient. In present case, the 10 mg L^{-1} Zn^{2+} was also selected to optimize the volume of CHI-CNDs-*E. coli*@TH mixed solution dropped on surface of GCE (d = 5 mm). As shown in Fig. 4b and Table S2 (P value < 0.05), when the volume changed from 30 μL to 15 μL, the inhibition rate of Zn^{2+} was continuously increased; however, as the droplet coating amount continued to decrease to 10 μL, the inhibition rate of Zn^{2+} was getting smaller, indicating that the highest inhibition rate could be obtained when the drop volume was 15 μL.

As discussed above, the parameters of microbial (*E. coli*) concentration and mixed solution volume of CHI-CNDs-*E. coli*@TH dropped on surface of GCE were studied and the optimal experiment conditions, i.e., the *E. coli* concentration OD_{600} = 2.5 and volume of CHI-CNDs-*E. coli*@TH 15 μL can generate the most sensitive current response.

3.3. Storage stability of IME

It is essential to investigate the storage time and ensure the stable activity of IME. Although the IME can be stored at low temperature to maintain the activity of *E. coli*, it can also cause apoptosis of microorganisms once the storage period is too long. To

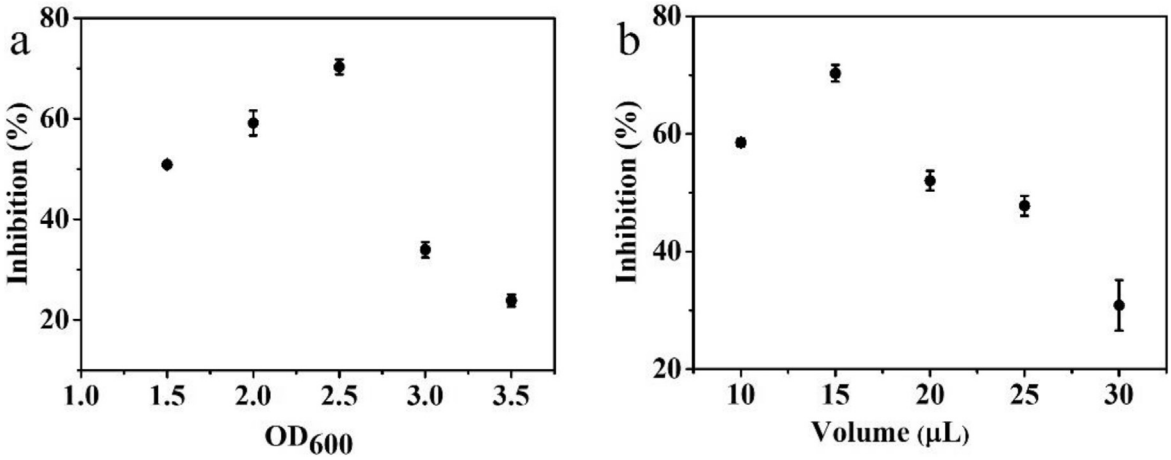

Fig. 4. Effect of (a) *E. coli* concentration and (b) volume of CHI-CNDs-*E. coli*@TH mixed solution dropped on the surface of glassy carbon electrode on toxicity assessment to10 mg L^{-1} Zn^{2+}.

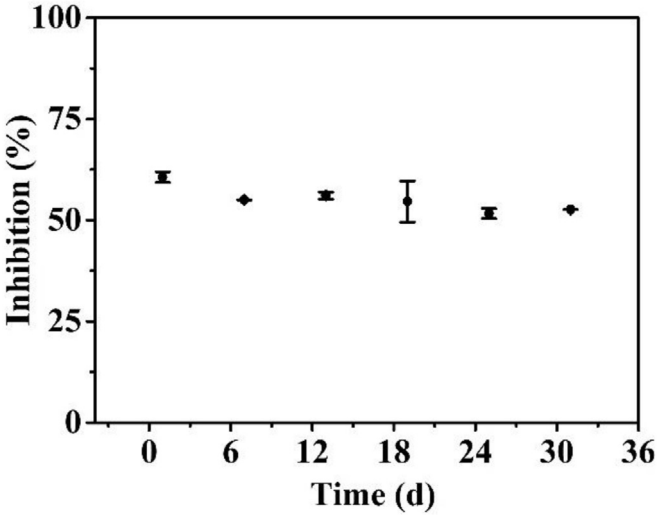

Fig. 5. The IME stability with different storage time at 4 °C.

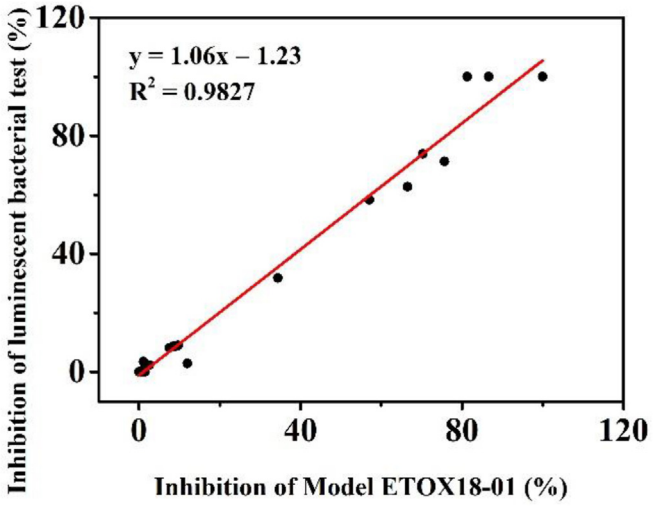

Fig. 6. Regression line between the results of Model ETOX18-01 instrument and luminescent bacterial test for various real and artificial water samples.

explore the storage stability, the same batch of IME was prepared and stored at 4 °C, and they were taken out every six days for the biotoxicity detection by using 10 mg L^{-1} Zn^{2+} as test toxicant. As shown in Fig. 5 and Table S3, the peak current of IME decreased slightly with time prolong. This may due to part of the *E. coli* in IME lost their metabolic activity. However, it did not significantly influence the detection result of inhibition rate. The results of toxicity detection remained in a small fluctuation range, and the RSD was 5.22% during the period of thirty-one days. It suggests that the IME can retain remarkable detection sensitivity and stabilization within certain period. This result is meaningful for the IME to realize batch production.

3.4. Assessment of real water samples by model ETOX18-01

The feasibility of the instrument above in water quality monitoring was tested based on measuring the toxicity of eighteen real water samples and six artificial wastewater samples. There were two river samples, two reservoir samples, two lake samples, four industrial wastewater samples, two laboratory wastewater samples, two soil leachate samples, four dining wastewater samples, two pesticide samples, two phthalic acid ester samples and two vapor organic mixture samples. The samples were analyzed contrastively by both luminescent bacterial test and the present Model ETOX18-01 instrument, and the results were shown in Table S4. The correlation between the two methods' results was given in Fig. 6. The Model ETOX18-01 instrument showed good correlation with the luminescent bacterial test, with the determination coefficient $R^2 = 0.9827$, suggesting that the present method could achieve the equivalent sensitivity with the ISO luminescent bacterial test. The results demonstrate that the developed mediated electrochemical biosensor method is promising and suitable for the biotoxicity determination of various ground surface water and wastewater.

3.5. Application of model ETOX18-01 in colored water samples detection

Generally, there are limitations for luminescent bacterial test regarding colored samples, such as some metal ions water samples, the color of which will interfere with emission wavelength of luminescent bacteria. However, the electrochemistry biosensor can make up for this shortcoming to some extent. In present work, metal ions water samples of Cu^{2+} and $Cr_2O_7^{2-}$ were selected and evaluated the inhibition rate by both luminescent bacterial test and Model ETOX18-01 instruments, the results were shown in Fig. 7 and Table S5 (P value < 0.05).

As for the colored samples, the inhibition rate of Model ETOX18-01 obtained had a linear dependence on the detected concentrations (Fig. 7a and b). However, for Cu^{2+}, the relationship between concentration and inhibition rate was irregular when detected by luminescent bacterial test. The reason is that the color of the sample is blue, whose wavelength range is 450–480 nm and overlaps with the luminescent bacteria's emission wavelength 450–490 nm. Furthermore, for $Cr_2O_7^{2-}$, a primary carcinogen, compared the IC_{50} = 0.47 mg L^{-1} obtained by Model ETOX18-01 (Fig. 7b), the inhibition value was obtained at a much higher concentration (IC_{50} = 12.48 mg L^{-1}) by luminescent bacterial test. Thus, the sensitivity of the electrochemical biosensor is better than that of luminescent bacteria method for $Cr_2O_7^{2-}$ detecting. The relative standard deviation (R.S.D.) and detection limits (LODs) were also utilized to represent the analytical characteristics of electrochemical biotoxicity early warning. The R.S.D values were 2.30%, 3.46% and the LODs values were 0.14 mg L^{-1}, 0.025 mg L^{-1} for Cu^{2+}, $Cr_2O_7^{2-}$, respectively.

4. Conclusion

In this work, we have developed an electrochemical biotoxicity early-warning instrument, Model ETOX18-01, and realized the detection of water samples of rivers, lakes, reservoirs, industrial water, dining wastewater, soil liquid and laboratory wastewater. The results obtained from these multiple wastewater samples assessment verify that the presented electrochemical biosensor method is comparable with the conventional ISO standardized luminescent bacterial test. Furthermore, the Model ETOX18-01 instrument integrates various functions including automatic cleaning, stirring, temperature control and data analysis, which provides a promising tool for aquatic environment assessment and early risk warning. In the future, further studies will be performed on more broad-spectrum sensitivity improvement and development of online electrochemical water toxicity detection.

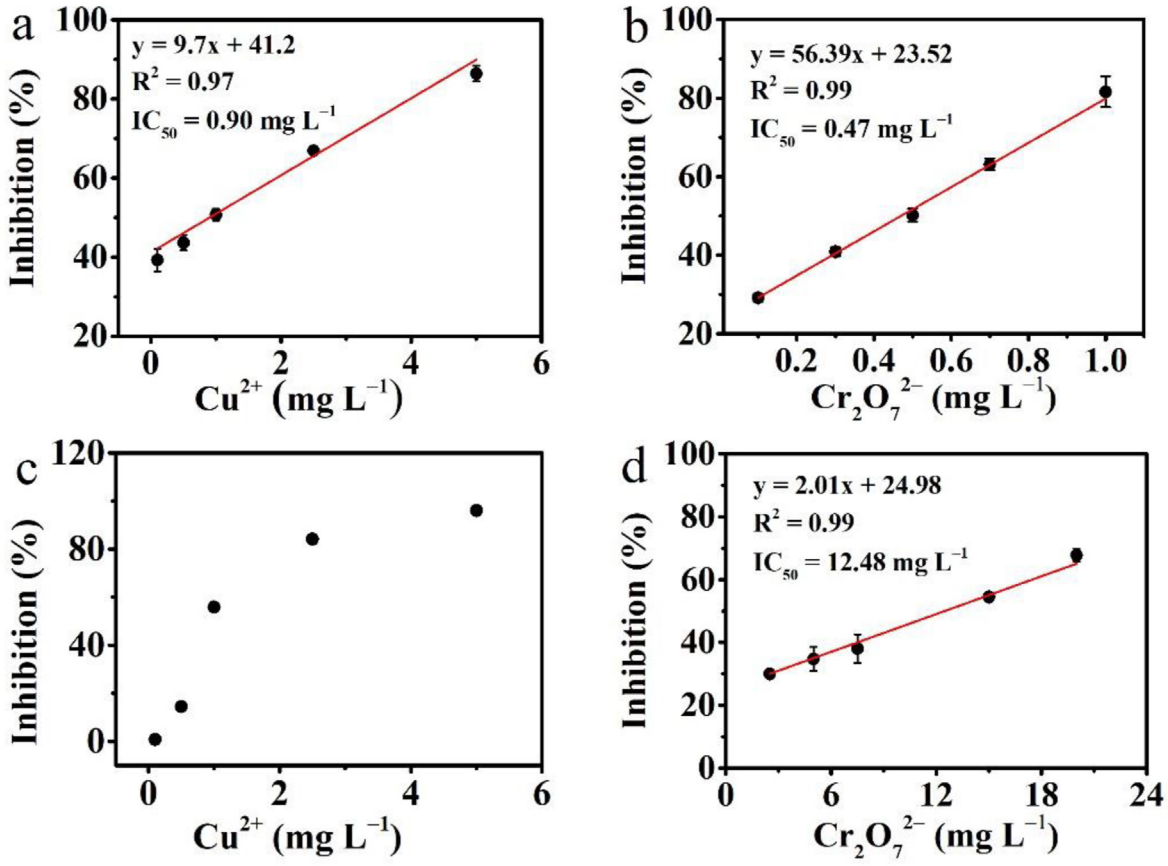

Fig. 7. Concentration–inhibition linear curves obtained by Model ETOX18-01 for (a) Cu^{2+}, (b) $Cr_2O_7^{2-}$, and luminescent bacterial test for (c) Cu^{2+}, (d) $Cr_2O_7^{2-}$, respectively.

Notes

The authors declare no competing financial interest.

Declaration of competing interest

The authors declare no competing financial interest.

CRediT authorship contribution statement

Yajie Yang: Conceptualization, Methodology, Data curation, Investigation, Software, Writing - original draft. **Yanran Liu:** Methodology, Formal analysis, Visualization, Writing - review & editing. **Yafei Chen:** Validation, Formal analysis, Visualization. **Yu Wang:** Project administration, Resources. **Peng Shao:** Project administration, Resources. **Runze Liu:** Formal analysis, Software. **Guanyue Gao:** Methodology, Supervision, Writing - review & editing. **Jinfang Zhi:** Funding acquisition, Resources, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.126964.

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