Analytica Chimica Acta 1228 (2022) 340354

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Development of portable whole-cell biosensing platform with lyophilized bacteria and its application for rapid on-site detection of heavy metal toxicity without pre-resuscitation

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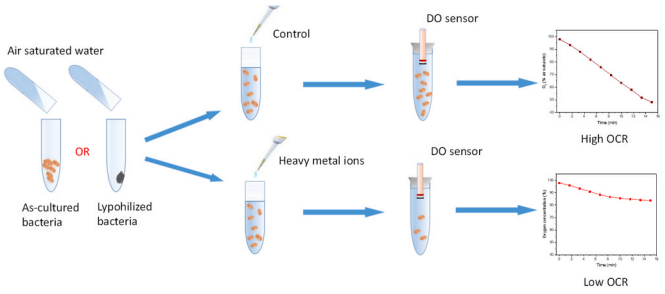
HIGHLIGHTS

- Innovative portable whole-cell biosensing platform was developed by combining DO sensor with culture/lyophilized bacteria.
- Integrated handheld optic fiber DO sensor was developed for DO detection in low volume sample (<1.0 mL).
- Using oxygen consumption inhibition mechanism, this platform achieved rapid acute toxicity detection of heavy metal ions.
- Lyophilized *E. coli* was directly applied for toxicity detection of heavy metal ions without pre-resuscitation in 18 min.
- Our strategy is fast, portable, storable, and suited for on-site ready-to-use and high-frequency toxicity detection.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Whole-cell biosensor
Toxicity detection
Heavy metal ions
Dissolved oxygen sensor
Lyophilized bacteria

ABSTRACT

The high toxicity of heavy metals necessitates monitoring technology that allows rapid adaptation and deployment. Microbial whole-cell biosensors have become a priority because of their excellent performance. However, traditional methods have several limitations, including long assay time, poor portability, and the lack of ready-to-use on-site devices. In this study, a novel portable whole-cell biosensing platform was developed by integrating a simple handheld fiber-optic dissolve oxygen sensor and bacterial culture or lyophilized bacteria. Based on the oxygen consumption inhibition mechanism, this platform achieved rapid acute toxicity measurement of heavy metal ions through inhibit *Escherichia coli* (*E.coli*) respiration. Under the optimal conditions, the limit of detection and IC50 of Hg^{2+} using *E. coli* culture were 5.62 μM and 11.64 μM, respectively. Interestingly, the lyophilized *E. coli* could be directly applied for Hg^{2+} toxicity detection without pre-resuscitation, where an

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https://doi.org/10.1016/j.aca.2022.340354
Received 29 April 2022; Received in revised form 26 July 2022; Accepted 2 September 2022
Available online 7 September 2022

IC50 of 31.28 μM was obtained, and the whole detection process was only 18 min. The lyophilized *E. coli* could be stored long-term at −80 °C without significant loss of activity and detection performance. The portable whole-cell biosensing platform demonstrated a high potential for rapid on-demand toxicity detection in real water samples. The developed strategy is not only fast, portable, and easily storable, but also highly suited for on-site ready-to-use, and high-frequency toxicity detection of heavy metal ions in the field.

1. Introduction

Heavy metals, which are ubiquitous environmental pollutants, are highly toxic, non-biodegradable, bio-accumulative, and potentially fatal [1–3]. Many countries and organizations have set maximum permissible exposure standards for heavy metals [2,4]. Rapid and high-frequency measurements are crucial for progression monitoring and timely treatment of contaminant accidents. Traditional analytical methods, such as inductively coupled plasma mass spectrometry, atomic absorption spectroscopy, and inductively coupled plasma atomic emission spectrometry, are highly sensitive and accurate [5,6]. However, it is difficult to determine the bioavailability and acute toxicity of heavy metal ions in living system using these methods [2,7]. Moreover, these techniques require expensive instrumentation, complex sample preparation, and long assay time. Accordingly, various methods based on exposure tests using organisms, including fish, shrimp, and amphipod, have been widely applied for acute toxicity detection [8–10]. However, they are generally time-consuming, costly, non-portable, and they cannot meet the requirements for a rapid emergency response to accidents.

As a supplement, microbial whole-cell biosensors have been used to measure pollutant toxicity (e.g., heavy metal ions) because they are easy to culture, cost-effective, and respond rapidly to external stimuli as bioreceptors without ethical risks [10,11]. Among all commercially available products, the most attractive mechanism for toxicity detection involves the metabolic activity of bioluminescent bacteria [12,13]. However, one of the main drawbacks of using bioluminescent bacteria is that samples with turbidity, absorbed light or quenched luminescence effects inevitably interfere with the analysis [11,14,15]. Furthermore, many bioluminescent bacteria require hyperosmotic and high-salinity environments, which may influence the solubility, bioavailability, and speciation of heavy metal ions, leading to inaccurate results [10,15]. Hence, a more rapid, portable, economical, and ready-to-use biosensor is highly required to achieve on-site high-frequency toxicity assessment of heavy metal ions in the field.

Molecular oxygen is a vital metabolite of bacteria and higher organisms, and changes in oxygen uptake can be a valuable marker of their metabolic status and responses to exogenous or endogenous stimuli [16,17]. Based on the respiration inhibition (or oxygen consumption inhibition) mechanism, microbial whole-cell biosensors, which are especially sensitive to various heavy metals, have been successfully applied for toxicity assessment, [11,16,17]. In these systems, the rapid and accurate measurement of dissolved oxygen (DO) is essential for the performance of microbial whole-cell biosensors. Fluorescence-based DO sensors are convenient and versatile tools because of their outstanding features [17]. Nevertheless, for their application in rapid on-site toxicity detection of heavy metal ions, a microbial whole-cell biosensor has to satisfy several requirements: (1) the bacterial cells must maintain long-term viability, activity and stability during storage and transport to the field site; (2) the oxygen consumption inhibition caused by a response to toxicity must be detected with a minimal interference from factors other than microbial respiration; and (3) ready-to-use assay need to apply the microbial whole-cell biosensor to detect real samples in the field. However, the existing microbial whole-cell biosensors are difficult to satisfy all of these requirements [10,16,17].

In this study, a novel portable microbial whole-cell biosensing platform was constructed to assess the toxicity of heavy metals by integrating a handheld fiber-optic DO sensor and lyophilized bacteria based on respiration inhibition mechanism. To achieve the real-time and accurate detection of DO, an integrated handheld fiber-optic DO sensor was developed based on an all-fiber structure and the phase-shift measurement principle. In this device, a new multimode optical fiber bundle was used for the simultaneous transmission of excitation light and fluorescence. The fiber optode was fabricated by coating an O_2 sensing foil at the end of the optical fiber bundle, and its size was small enough to allow *in situ* DO detection in a small volume sample (<1.0 mL). As previously reported, lyophilization is an effective method for maintaining long-term activity and stability of bacteria thanks to the lack of cellular growth [18]. Here, an effective bacterial lyophilization method was proposed that could be directly applied for ready-to-use assays of heavy metal toxicity without pre-resuscitation.

2. Experimental

2.1. Materials and reagents

The materials and reagents, including the cultivation and lyophilization of *E. coli*, are shown in the Supporting Information.

2.2. Handheld fiber-optic DO sensor

To achieve rapid *in situ* detection of DO, we developed a simple, cost-effective, and integrated handheld optic fiber DO sensor based on an all-fiber structure and the phase-shift measurement mechanism (Fig. 1A). In this device, a multimode optical fiber bundle was employed for the transmission of excitation light and the collection and transmission of fluorescence, which greatly simplified DO sensor structure and improved the optical transmission efficiency. An O_2-sensing foil containing fluorescence oxygen-sensitive probe (PtTFPP) was coated on the end of the optical fiber bundle to construct the fiber optode. This elegant optical design enables high portability and stability, because it does require other optical separation elements.

It is well known that the fluorescence signal exhibits a delay phase-shift relative to the excitation signal (or reference light signal) that can be measured, and this shift changes with the oxygen concentration as follows (O·S [19].:

$$\frac{I_0}{I}=\frac{\tau_0}{\tau}=1+K_{sv}[O_2] \quad (1)$$

where I_0, I, τ_0, and τ are the fluorescence intensities and lifetimes in the absence and presence of oxygen [O_2], respectively. K_{sv} is the Stern-Volmer constant. The fluorescence probe is reversibly quenched by O_2, whose depletion due to bacterial respiration causes a change in the fluorescence intensity and phase-shift, thus allowing continuous monitoring and real-time quantification of DO in water. Because the phase-shift measurement was not affected by fluorescence intensity, the effects of sample turbidity, absorbed light, or quenched luminescence could be avoided. Furthermore, the handheld optical fiber DO sensor allowed the real-time *in situ* detection of DO in samples with a low volume (<1.0 mL) because of the small diameter (<2.0 mm) of the optode (Fig. 1B).

2.3. Portable microbial whole-cell biosensing platform and toxicity detection mechanism

The portable microbial whole-cell biosensing platform (p-MWB) consisted of handheld optic fiber DO sensor and bacterial culture or

lyophilized *E. coli* (Fig. 1C); the measurement mechanism is shown in Fig. 1D. The oxygen consumption originating from *E. coli* respiration was indicative of its metabolic state, which was measured using a handheld fiber-optic DO sensor. In the absence of toxic substances, *E. coli* consumes oxygen at a higher rate, which depends on its concentration. The addition of heavy metal ions causes *E. coli* respiration inhibition, thus resulting in a decreased oxygen consumption rate (OCR). The OCR is defined as the oxygen saturation decrease value per minute. Inhibition of the OCR, *I*, is calculated by comparing the OCR of a real sample with a control sample containing no toxic substance, which is used for toxicity assessment and given as,

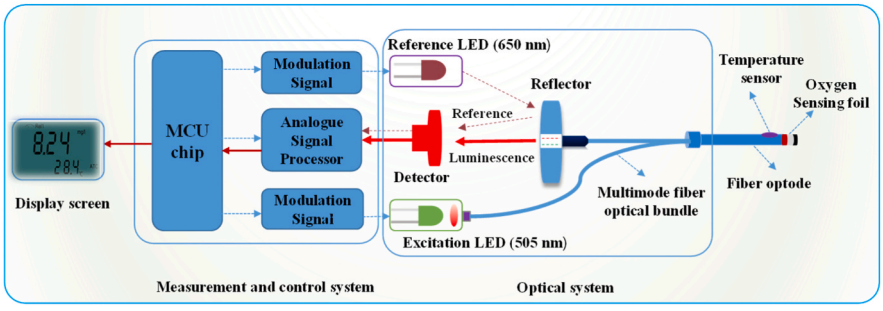
$$I = 1 - \frac{R_s}{R_b} \times 100\% \quad (2)$$

where R_b is the OCR in the absence of toxic heavy metal ions and is calculated as:

$$R_b = (RO_{b0} - RO_{bt})/t \quad (3)$$

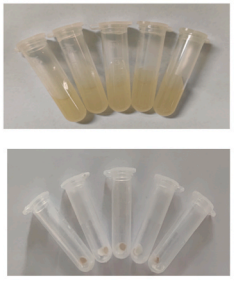
Where RO_{b0} is the initial oxygen saturation, and RO_{bt} is the oxygen saturation after *t* min. R_s is the OCR in the presence of toxic heavy metal ions and is calculated as:

$$R_s = (RO_{s0} - RO_{st})/t \quad (4)$$

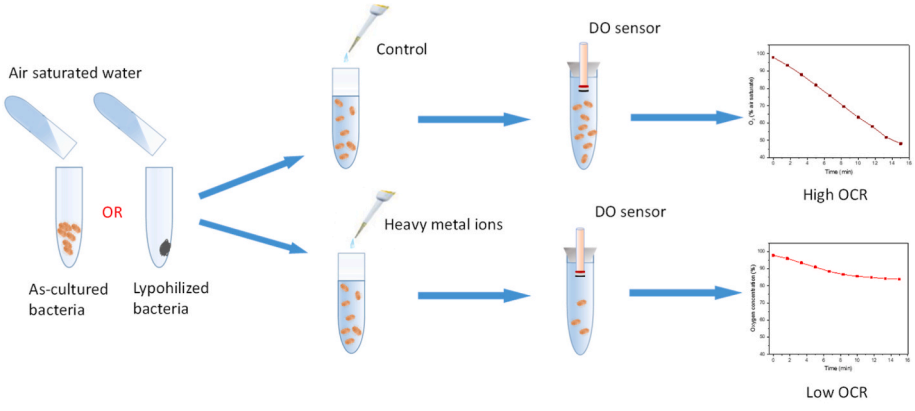


(A)

(B)



(C)



(D)

Fig. 1. (A) Schematic illustration of the handheld fiber-optic DO sensor, consisting of a compact optical system, measurement and data processing system, a mini-temperature sensor, and display screen. (B) Image of handheld optical fiber DO sensor. (C) *E. coli* culture (up) and lyophilized *E. coli* (down). (D) Schematic diagram of the mechanism for toxicity detection based on *E. coli* culture or lyophilized *E. coli* using the portable microbial whole-cell biosensing platform.

Where RO_{s0} is the initial oxygen saturation, and RO_{st} is the oxygen saturation after t min.

2.4. Toxicity test

The OCR inhibition method, similar as ISO 8192 (ISO 2007), has been applied for toxicity detection of heavy metal ions [17]. First, the oxygen saturation change of air-saturated water with a certain concentration of *E. coli* was tested as a control. Then, 0.5 mL of *E. coli* cultured overnight and a 0.5 mL heavy metal ion solution of a certain concentration were added to 4 mL air-saturated water, and the DO was immediately measured using the handheld optical fiber DO sensor. After a certain reaction time, OCR inhibition can be determined according to Eq. (2), which is used to assess heavy metal toxicity.

When lyophilized *E. coli* were used for toxicity measurement of heavy metal ions, 4.0 mL air-saturated water and 1.0 mL samples containing various concentrations of heavy metal ions were added into a sample tube. DO was real-time detected using handheld optical fiber DO sensor, which was used for toxicity assessment of heavy metal ions.

3. Results and discussion

3.1. Characteristics of portable microbial whole-cell biosensing platform

The performance of the DO sensor is essential for the proposed microbial whole-cell biosensing platform based on the oxygen consumption inhibition mechanism. The developed handheld fiber-optic DO sensor is based on the dynamic molecular oxygen quenching of the PtTFPP fluorescence probe because of its excellent photostability and insensitivity to temperature [20]. As described in a previous study, the relationship between phase-shift and oxygen saturation (or DO) can be described as following [21].

$$\frac{Tan(\varnothing_0)}{Tan(\varnothing_0)-Tan(\varnothing)}=\frac{1}{f_1\cdot K_{sv}}\cdot\frac{1}{[O_2]}+\frac{1}{f_1} \quad (5)$$

where K_{sv} is the Stern-Volmer constant, φ_0 is the original phase-shift in the absence of oxygen, and φ is the fluorescence phase-shift that changes with the oxygen concentration, f_1 and f_2 represent the fraction of the fluorescence probe that is quenched by oxygen and the fraction that cannot be quenched, respectively, and the sum of f_1 and f_2 is 1. We established a relationship between the phase-shift of the handheld fiber-optic DO sensor and the oxygen saturation (or DO concentration) (Fig. 2A), where the oxygen saturation in water was adjusted from 100% to 0% using calibration gases with different oxygen concentrations. Fig. 2A shows a good linear relationship between $\frac{Tan(\varnothing_0)}{Tan(\varnothing_0)-Tan(\varnothing)}$ and $\frac{1}{[O_2]}$ with a slope of 27.8489, and $R^2 = 0.9999$. Thus, the oxygen saturation can be determined. Fig. S1 shows the continuous monitoring of oxygen saturation in a water sample in a closer tube using the proposed DO sensor. There was no obvious change (<2%) in oxygen saturation over a long time (>10 h). These results indicate that the proposed DO sensor is highly stable. Because the size of the DO sensor is very small (<φ 2 mm), it is very suitable for DO detection in a small volume sample.

E. coli was used as the sensing agent for real-time *in situ* OCR testing due to its ability of to undergo both anaerobic and aerobic respiration [22]. To observe the OCR, an overnight culture of *E. coli* was diluted with air-saturated water to 5.0×10^7 cfu/mL and loaded into a 5.0 mL sample tube kept at 37 °C in a home-made incubator. Oxygen saturation in the sample tube was recorded using a handheld fiber-optic DO sensor every 2 min. The oxygen saturation in water sample decreased over time (red line in Fig. 2B). As a control, the concentration of DO remained unchanged when no *E. coli* was added, which contributed to the advantages of fluorescence-quenched based DO sensor, such as the lack of oxygen consumption and high stability. Therefore, oxygen consumption should originate from bacterial respiration and reproduction, because all test processes were carried out in a closed test tube.

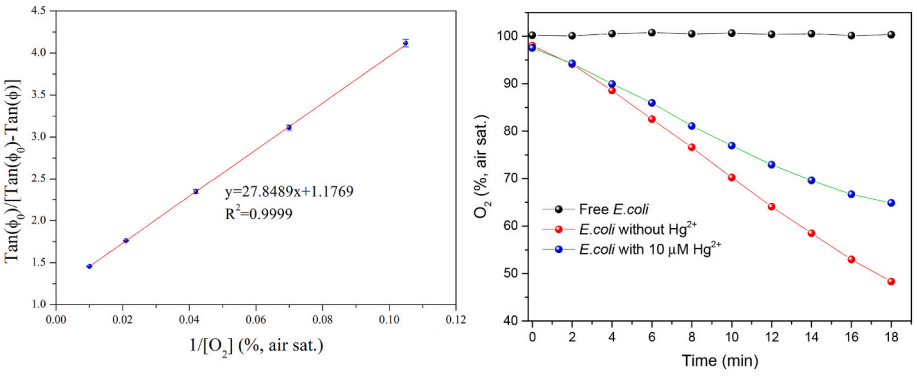
Finally, the feasibility of the proposed microbial whole-cell biosensing platform for detecting the acute toxicity of heavy metal ions was verified. The toxic effects of Hg^{2+}, a representative heavy metal ion, on the *E. coli* activity were examined. As shown the green line of Fig. 2B, after the *E. coli* and 10 μM Hg^{2+} were simultaneously added to air-saturate water, the decrease value of oxygen saturation was significantly lower than without Hg^{2+}. This contributes to the respiratory activity inhibition of *E. coli* due to Hg^{2+} addition, thus resulting in a decrease of OCR. This verified that the proposed biosensing platform could be applied to measure the acute toxicity of heavy metal ions.

3.2. Optimization of testing conditions

Several testing conditions were optimized before the subsequent experiments. *E. coli* concentration is a key factor in determining the performance of whole-cell biosensors [22]. To determine the optimal *E. coli* concentration, a sensitivity index (ε) was introduced as follows:

$$\varepsilon = R_s/R_b \quad (6)$$

Where R_b and R_s are the OCRs without heavy metal ions and with heavy metal ions, respectively. Hg^{2+} was selected as a standard toxicant to evaluate the response of the microbial whole-cell biosensor. Experiments were performed using serial dilutions of *E. coli* (Fig. S2A). Increasing *E. coli* concentration resulted in an increase of the OCRs. The time required to switch from sample air-saturated to deoxygenated conditions was the shortest (less than 20 min) when the *E. coli* concentration was 1.0×10^8 cfu/mL. When 20 μg/L Hg^{2+} was introduced into the water samples, the OCR of all samples decreased (Fig. S2A). OCR inhibition improved with increasing *E. coli* concentrations (Fig. S2B).



(A) (B)

Fig. 2. Characteristics of portable microbial whole-cell biosensing platform. (A) The relationship between oxygen saturation and phase-shift of the handheld fiber-optic DO sensor at 37 °C. (B) The typical traces of oxygen saturation in different water samples over time. Black line: no *E. coli* and Hg^{2+}; Red line: adding 5.0×10^7 cfu/mL *E. coli* but no Hg^{2+}; Blue line: adding 5.0×10^7 cfu/mL *E. coli* and 10 μM Hg^{2+}. Testing conditions: pH = 7 and 37 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

This is because microbial cell respiration was increasingly inhibited with increasing concentration at the same toxicant concentration, which is consistent with previous reports [16,22]. Although a higher sensitivity index was obtained at 1.0×10^7 cfu/mL, a relative higher *E. coli* concentration (5.0×10^7 cfu/mL) was chosen as the optimal concentration for subsequent toxicity measurements considering the on-site rapid detection requirements and cell culture conditions. To shorten the measurement period, the testing time was set as 18 min if no special statement.

Bacteria metabolic activity is highly dependent on temperature. Fig. S2C shows the oxygen saturation changes of the samples (pH = 7.0) containing 5.0×10^7 cfu/mL *E. coli* at different temperatures. Oxygen consumption was very slow at relatively low temperatures (<20 °C), because the metabolic activity of *E. coli* was reduced. With an increase in temperature, the *E. coli* OCR gradually increased, and the maximum OCR was obtained at ~40 °C (Fig. S2C). However, a higher temperature (>40 °C) suppressed the metabolic activity of microbial cells, resulting in a low OCR. Although the highest respiratory activity could be obtained at ~40 °C, hydrolysis of heavy metals might be occurred at such high temperatures. Thus, a suitable incubation temperature of 37 °C was selected for toxicity test.

pH is another important factor that affects bacterial metabolic activity. The oxygen consumption profiles of *E. coli* at different pH values are shown in Fig. S2D. The chart clearly shows that the OCR significantly increases with an increasing in pH when the pH is less than 7.0. In the pH range of 7.0–11.0, a decrease in OCR was observed with a further increase in pH, which demonstrated the evident negative effect. These results clearly indicate that the respiratory activity of *E. coli* is pH-sensitive. The test pH (7.0) was used in subsequent experiments. Both pH and temperature had significant effects on *E. coli* metabolic activity. Therefore, the potential inhibitory effects of pH and temperature should be excluded before evaluating heavy metal ion toxicity.

3.3. Hg^{2+} toxicity detection using bacterial culture

Once the optimal assay parameters were determined, acute Hg^{2+} toxicity detection was performed using the portable microbial whole-cell biosensing platform. The *E. coli* culture were diluted to 5.0×10^7 CFU/mL using air-saturated water. After the addition of a 0.5 mL Hg^{2+} solution, leading to a final Hg^{2+} concentration of 0–22.0 μM, oxygen saturation was real-time detected using the handheld fiber-optic DO sensor. Typical oxygen consumption traces of *E. coli* treated with various Hg^{2+} concentrations are shown in Fig. 3A. The OCRs of the treated samples containing Hg^{2+} decreased significantly and were lower than those of the control sample without Hg^{2+}. This contributed to the inhibited *E. coli* respiration by toxic Hg^{2+} via inhibition of metabolic activity, which could limit cellular activity and even lead to cell death. This was verified using the traditional plate counting method (Fig. S3). When Hg^{2+} concentration was higher than 400 nM, no *E. coli* could grow in LB medium. However, we could still observe oxygen consumption in experimental concentration range of Hg^{2+}, which was much higher than 400 nM. This is because the interaction time between Hg^{2+} and *E. coli* lasted more than 12 h in the plate counting method, and a lower concentration of Hg^{2+} could lead to *E. coli* death. However, in our proposed method, the interaction time was very short (<20 min), the metabolic activity of *E. coli* was maintained, and their respiration was not completely suppressed.

Fig. 3B shows the dose-response curve of Hg^{2+} on *E. coli* respiration inhibition, which was plotted the OCRs against Hg^{2+} concentration using a four-parameter model (Supporting Information). The error bars in Fig. 3B correspond to the standard deviation of the data points in triplicate experiments, and all of them were less than 6.3%, indicating good stability of the portable microbial whole-cell biosensor for Hg^{2+} toxicity detection. From Fig. 3B, the limit of detection (LOD) determined for Hg^{2+} using three times the standard deviation of the mean blank values was 5.62 μM, and the IC50 value was 11.64 μM. Table S1 compares the relative toxicity values of Hg^{2+} using the proposed biosensor with other biosensors under comparable conditions [22–26]. It can be seen that sensitivity of the proposed bioassay is comparable with those of the other methods (Table S1).

3.4. Towards a universal acute toxicity detection platform

To demonstrate the general applicability of the portable whole-cell biosensing platform, the toxicities of other three heavy metals, including Pb^{2+}, Zn^{2+}, and Cu^{2+}, were also assessed via bacterial culture under the optimal conditions. Their detection processes were similar to that of the Hg^{2+}. The *E. coli* concentration was 5.0×10^7 CFU/mL, and the detection time was 18 min. Fig. 4A–C shows the typical oxygen consumption profiles at different heavy metal ion concentrations. As expected, increasing the concentration of heavy metal ions led to a proportional decrease of OCRs.

Fig. 4D shows the dose-response curves of three heavy metal ions in *E. coli* respiration inhibition, which was plotted the OCR against the concentrations of Pb^{2+}, Zn^{2+}, and Cu^{2+}, respectively. The IC50 values of Pb^{2+}, Zn^{2+}, and Cu^{2+} were 323.6, 565.0, and 859.2 μM, respectively, which were higher than that of Hg^{2+}. The toxicity of four heavy metal ions can be ranked in a descending order: $Hg^{2+} > Pb^{2+} > Zn^{2+} > Cu^{2+}$, based on the measured IC50 values. The IC50 values of three metal ions were highly comparable with those of the other methods [22,26–28].

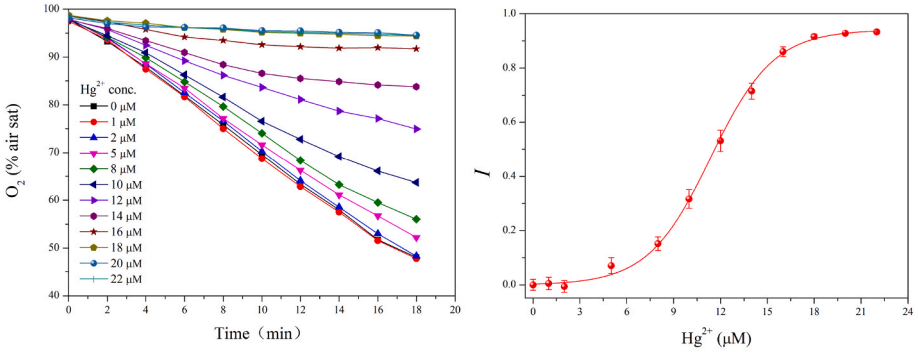


Fig. 3. Toxicity detection of Hg^{2+} using the portable microbial whole-cell biosensing platform. (A) Typical oxygen consumption traces at various Hg^{2+} concentrations. (B) Dose-response curve of Hg^{2+} toxicity detection. The *E. coli* concentration is 5.0×10^7 cfu/mL, pH = 7, and the temperature is 37 °C.

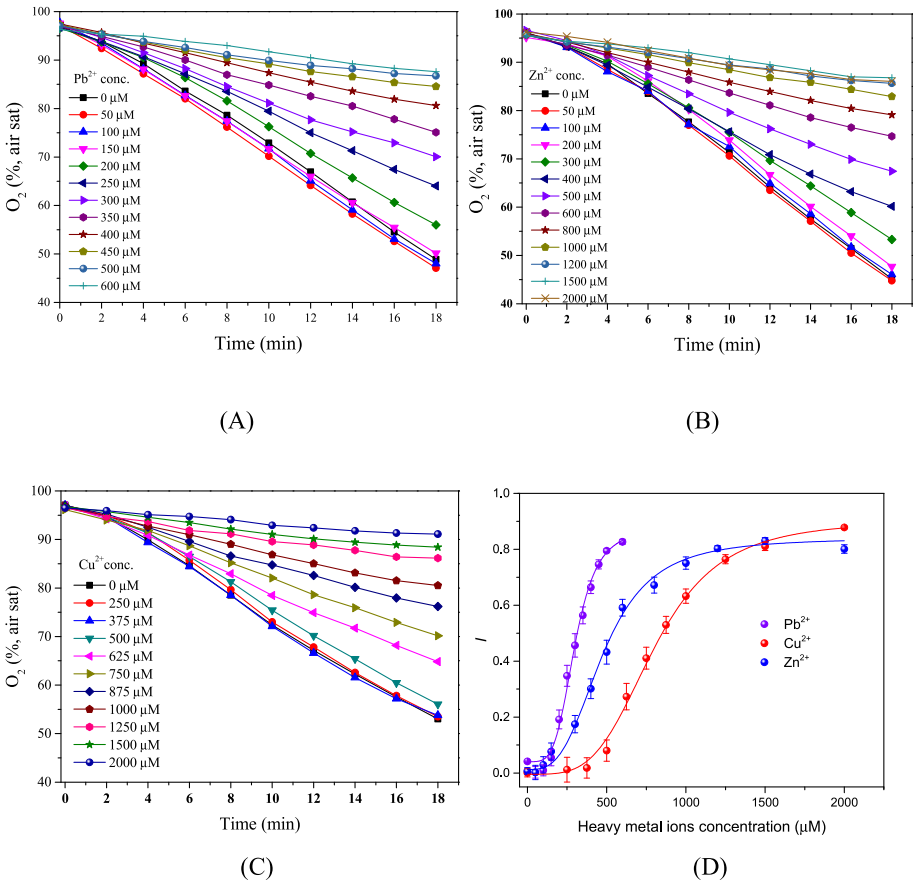


Fig. 4. Toxicity detection of Pb^{2+}, Zn^{2+} and Cu^{2+} using portable microbial whole-cell biosensing platform. (A–C) The typical oxygen consumption traces at various concentrations of Pb^{2+}, Zn^{2+} and Cu^{2+}, respectively; (D) The dose-response curves of toxicity detection of three heavy metal ions. The *E. coli* concentration is 5.0×10^7 cfu/mL, pH = 7, and the temperature is 37 °C.

The other methods generally required a longer analysis time; conversely, the proposed method required only 18 min. The above results show the scalability of the proposed microbial whole-cell biosensor for biological hazard and toxicity analysis of heavy metal ions.

Monitoring the respiration of model organisms and studying changes in their metabolism induced by external toxicants allows for the assessment and ranking of heavy metal ion toxicity [11,16,17]. Except for this, the proposed method embraces several other advantages. First, the miniaturization, mobility, and flexibility of portable microbial whole-cell biosensor benefits for rapid (18 min) on-site toxicity detection of heavy metal ions with simple operation. Second, on-site high--throughput toxicity detection of heavy metal ions can be achieved without the use of expensive instruments and reagents. In actual applications, only the end-detection of DO is required, and our proposed method is feasible for high-frequency and high-throughput toxicity detection of heavy metal ions, which is beneficial for early-warning and rapid detection of heavy metal pollution. Third, the portable microbial whole-cell biosensing platform was very cheap (only $ 300), and was as little as $ 0.05 per test. Therefore, it can be afforded by the developing countries and resource-limited settings. In addition, the toxicity detection of heavy metal ions based on the respiration activity of microbial cells is not affected by the turbidity, absorbed light or quenched luminescence effect of the samples, benefiting for obtaining a more accurate result.

3.5. Hg^{2+} toxicity detection using lyophilized bacteria

The long-term activity and viability of whole-cell biosensors are vital for the development of cell-based devices and their widespread practical application in routine environmental monitoring [11,16,18], especially in remote, low-resource settings. Lyophilization is one of the most effective methods for long-term preservation of the bacteria because of the lack of cellular growth, and it is also beneficial for ready-to-use detection [18,29].

The toxicity detection mechanism of Hg^{2+} using lyophilized bacteria was similar to that of bacterial culture, except for the use of lyophilized *E. coli* (Fig. 1D). 4.0 mL air-saturated water was initially added to the sample tube for rehydration. After 1.0 mL control sample or sample containing Hg^{2+} was introduced, oxygen saturation was real-time detected using the handheld fiber-optic DO sensor. First, we evaluated the recovery performance of the lyophilized *E. coli*. Interestingly, the respiration of lyophilized *E. coli* rapidly recovered without pre-resuscitation after the introduction of air-saturated water (Fig. 5A). In particular, the oxygen in the water samples was completely depleted within 18 min, which contributed to good preservation of the activity and viability of *E. coli* in the samples. The plate counting method showed that the concentration of lyophilized *E. coli* prepared using air-saturated water was about 1.5×10^8 cfu/mL after recovery.

Lyophilized *E. coli* was used for Hg^{2+} toxicity detection. Typical oxygen consumption traces of *E. coli* treated with Hg^{2+} are shown in Fig. 5A. Similar to the bacterial culture, the OCRs of lyophilized *E. coli* significantly decreased when the water samples were spiked with Hg^{2+}. This demonstrates that the respiratory activity of lyophilized *E. coli* was also greatly inhibited by toxic Hg^{2+}. Higher concentrations of Hg^{2+} led to greater inhibition of *E. coli* respiration. Fig. 5B shows dose-response curve of Hg^{2+} on OCR inhibition. The error bars in Fig. 5B are less than 5.9%, indicating that the portable microbial whole-cell biosensing platform also had good stability for Hg^{2+} toxicity detection using

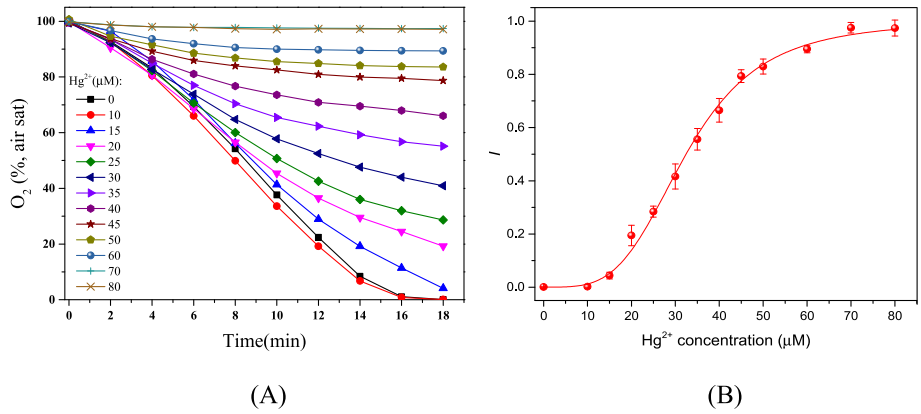


Fig. 5. Hg^{2+} Toxicity detection using the lyophilized *E. coli*. (A) Typical oxygen consumption traces at various concentrations of Hg^{2+}. (B) Dose-response curve of Hg^{2+} toxicity detection. The detection conditions: pH = 7, and the temperature is 37 °C.

lyophilized bacteria. The LOD and IC50 value determined for Hg^{2+} using lyophilized bacteria was 13.18 μM and 31.28 μM, respectively. The IC50 of lyophilized cells was slightly higher than that of bacterial culture, but they were also highly comparable with the previous reports [23–25]. These results clearly demonstrated that lyophilized *E. coli* could be applied for the rapid on-site toxicity detection of heavy metal ions without pre-resuscitation. This appearance was completely different from other reports that required overnight recovery [10,16,18]. To assess the stability and repeatability, we used the proposed cell biosensor for 10 times continuous detection of Hg^{2+}, a good consistent was obtained and the RSD was less than 10% (Fig. S4). These results demonstrated that the present method could greatly benefit for the ready-to-use application of biosensors.

Furthermore, maintaining the viability and sensitivity of a bacterial whole-cell biosensor is one of the most important features for evaluating its potential long-term functionality [16,18]. The performance of lyophilized *E. coli* was evaluated after long-term storage at 4 °C, −20 °C, and −80 °C. First, the respiration activity of lyophilized *E. coli* at three storage temperatures was measured after different storage durations (Figs. S5A–C). Fig. S5D shows the different OCRs of lyophilized *E. coli* depending on storage temperature. The OCRs of lyophilized *E. coli* decreased with the extension of storage time when stored at 4 °C and −20 °C. After a month of storage, the OCRs at 18 min decreased to 70.1% and 35.2% at 4 °C and −20 °C, respectively. However, when the lyophilized *E. coli* were stored at −80 °C, the OCR showed only a slight decrease (<5%), even after 1 month. Previous studies have indicated that residual water remaining in lyophilized culture might result in partial rehydration and inactivation of lyophilized cells during storage. Consequently, storage at −80 °C was better for preserving the activity of bacterial cells.

To investigate the performance of the lyophilized whole-cell biosensor over time, Hg^{2+} toxicity detection was performed using lyophilized *E. coli* after 15 and 30 days of storage at −80 °C. The dose-response curves are shown in Fig. S5E, compared with that obtained after 24 h of lyophilization as a benchmark reference. The IC50 values of lyophilized *E. coli* after 1 d, 15 d and 30 d storage were 29.5 μM, 30.8 μM, and 28.8 μM, respectively, with no significant change during the 1 month of storage.

3.6. Toxicity detection of real water samples

To explore the potential use of the portable microbial whole-cell biosensing platform in the field, the *E. coli* culture and lyophilized *E. coli* were applied for Hg^{2+} toxicity detection in real water samples, including tap water, river water (Gaoliang River, Being), lake water (Yishaochi Lake, Renmin University of China), and the effluent of a wastewater treatment plant (WWTP). The oxygen consumption ability of four water samples was initially investigated, because some organisms could survive in these samples. After four water samples were saturated using air, their DO concentration was real-time tested using the handheld fiber-optic DO sensor. As shown in Fig. S6, oxygen saturation in tap water was almost unchanged, but those in other three water samples slightly decreased (<5%) after 2 h, which should originate from the respiration of existing organisms. However, this slight decrease of oxygen saturation did not affect the measurement results because the toxicity detection of heavy metal ions was completed in 18 min, and the concentrations of *E. coli* culture or lyophilized *E. coli* were much higher than those of the existing organisms.

Figs. S7A and S7B present the dose-response curves of Hg^{2+} in respiration inhibition of *E. coli* in four water samples using *E. coli* culture and lyophilized E. coli, respectively. When using *E. coli* culture, the IC50 values of Hg^{2+} on *E. coli* were 10.35 μM, 12.47 μM, 11.24 μM, and 12.28 μM for tap water, river water, lake water, and the WWTP effluent, respectively. As expected, the IC50 values using lyophilized *E. coli*, were higher than those of the *E. coli* culture for all four water samples, and they were 35.12 μM, 37.46 μM, 42.35 μM, and 48.15 μM for tap water, river water lake water, and WWTP effluent, respectively. The IC50 value of tap water was slightly lower than those of the other three water samples. Differences of the bioassay performance in various water matrices might be related to their water chemistry characteristics, which could affect the pollutant bioavailability and toxicity, especially for heavy metals. Furthermore, the interactions between heavy metals and other pollutants in the water samples might cause lower or higher toxicity towards the *E. coli*. In summary, the proposed portable microbial whole-cell biosensing platform could directly detect the toxicity of heavy metal ions using *E. coli* culture or lyophilized *E. coli*. However, the use of lyophilized *E. coli* is not only beneficial for its long-term storage and cold-chain independence, but also readily reconstitutes for rapid on-site detection.

4. Conclusion

A novel portable microbial whole-cell biosensing platform was successfully constructed for ready-to-use toxicity assays of heavy metal ions by integrating a handheld fiber-optic DO sensor with bacterial culture or lyophilized bacteria. Toxicity detection of heavy metal ions could be rapidly achieved based on the oxygen consumption inhibition mechanism. Lyophilized *E. coli* could be directly applied for Hg^{2+} toxicity detection without pre-resuscitation, and the lyophilized *E. coli* could be long-term stored at −80 °C without significant loss of activity and detection performance. The portable whole-cell biosensing platform was robust and worked reliably with complex samples such as WWTP effluent or environmental water. For practical use, future efforts will focus on the performance optimization of the developed biosensing

platform and validating its application for on-site detection of large-scale environmental samples. In a word, the proposed microbial whole-cell biosensing platform is not only greatly suitable for the rapid high-frequency on-site toxicity assessment of heavy metal ions, but also is highly scalable because of its simplicity, speed, ready-to-use, long-term stability, and flexibility.

CRediT authorship contribution statement

Hongliang Wang: performing all the experiments and writing the manuscript. **Dan Song:** performing all the experiments and writing the manuscript. **Yuyang Chen:** drawing and summarizing figures. **Wenjuan Xu:** performing all the experiments and writing the manuscript. **Xiangzhi Han:** drawing and summarizing figures. **Anna Zhu:** designing, managing the project, and Editing, All the authors discuss the results and commented on the manuscript. **Feng Long:** drawing and summarizing figures, designing, managing the project, and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant No. 21675171).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2022.340354.

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