

# A mediator-free whole-cell electrochemical biosensing system for sensitive assessment of heavy metal toxicity in water

Yuan Yang, Zhen Fang, Yang-Yang Yu, Yan-Zhai Wang, Saraschandra Naraginti and Yang-Chun Yong

## ABSTRACT

A bioelectrochemical sensing system (BES) based on electroactive bacteria (EAB) has been used as a new and promising tool for water toxicity assessment. However, most EAB can reduce heavy metals, which usually results in low toxicity response. Herein, a starvation pre-incubation strategy was developed which successfully avoided the metal reduction during the toxicity sensing period. By integrating this starvation pre-incubation procedure with the amperometric BES, a sensitive, robust and mediator-free biosensing method for heavy metal toxicity assessment was developed. Under the optimized conditions, the IC<sub>50</sub> (half maximal inhibitory concentration) values for Cu<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>, and Cr<sup>6+</sup> obtained were 0.35, 3.49, 6.52, 2.48 mg L<sup>-1</sup>, respectively. The measurement with real water samples also suggested this method was reliable for practical application. This work demonstrates that it is feasible to use EAB for heavy metal toxicity assessment and provides a new tool for water toxicity warning.

**Key words** | bioelectrochemical system, heavy metals, *Shewanella*, toxicity assessment, water toxicity

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## INTRODUCTION

Heavy metal pollution in water systems is an urgent problem that seriously threatens the health of living creatures on the earth as heavy metals are non-biodegradable and bioaccumulative (Gumpu *et al.* 2015). Heavy metals can be easily accumulated in the biosphere, especially in the human body. Although trace heavy metals are essential to cell signalling, metabolism and enzymatic catalysis, these compounds can be harmful to living cells at higher concentrations (Singh *et al.* 2010). For example, Cu<sup>2+</sup> is considered as a carcinogen because of its inhibition on the electron transport chain and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity (Einicker-Lamas *et al.* 2002; Sheline & Choi 2004; Gumpu *et al.* 2015). As a result, the Cu<sup>2+</sup> concentration should be strictly controlled in drinking water (Wong *et al.* 2018). Therefore, it is important to develop an efficient, reliable, and cost-effective method for heavy metal toxicity assessment in water systems.

The biosensing method attracted much attention for biotoxicity assessment as it can provide the unique information of bioavailability. To date, various model organisms, such as zebrafish and algae, have been used

for biotoxicity monitoring as they are sensitive to environmental stimuli (Li *et al.* 2015; Islam *et al.* 2017; Wong *et al.* 2018). However, methods based on higher organisms usually encountered the problems of a time-consuming, complicated culture condition and high cost (van der Schalie *et al.* 2001; Farre & Barcelo 2003). Microorganisms, with the advantages of a fast growth, simple culture conditions and low cost, were considered as another alternative host for whole-cell biotoxicity assessment. In addition, microorganisms could achieve fast response to environmental toxicity because of their simple cell structure and metabolism (Wang *et al.* 2013b; Chen *et al.* 2014; Fang *et al.* 2016; Ahmed & Oh 2018). However, the problem encountered with the microbial biosensors is the signal transduction, i.e., how to transduce the biotoxicity to a readable output signal. Several signal transduction strategies have been established for microbial biosensors, including fluorescence, luminescence, enzyme activity, and electrochemical activity, (Vopalenska *et al.* 2015; Chen *et al.* 2016; Islam *et al.* 2017; Cui *et al.* 2018; Yang

*et al.* 2018b). However, transduction of the bio-signal into a readable electric signal with an electrochemical tool was proved as one of the most efficient and cost-effective approaches (Gumpu *et al.* 2015; Fang *et al.* 2016; Gao *et al.* 2016).

During the past decades, various whole-cell bioelectrochemical sensing systems have been developed and applied for heavy metal toxicity assessment (Wang *et al.* 2013b; Gumpu *et al.* 2015; Gao *et al.* 2016; Yang *et al.* 2018a; Zhang *et al.* 2018). However, these biosensors usually relied on the utilization of mediators (redox-active molecules) to transport the electrons between the electrode and cells (Gumpu *et al.* 2015). Strikingly, it was recently found that some electroactive bacteria (EAB) could exchange the electrons with electrode without an exogenously added mediator (Logan 2009; Liao *et al.* 2015). Compared with the exogenously added mediator-based bioelectrochemical sensing system (BES), the EAB-based mediator-free biosensor showed advantages of easy operation, cost-effectiveness and high sensitivity (Si *et al.* 2015; Fang *et al.* 2016; Yang *et al.* 2018a, 2018b). Thus, it is expected to use EAB to construct mediator-free whole-cell electrochemical biosensors for heavy metal toxicity assessment.

*Shewanella oneidensis* MR-1 is one of the most extensively studied model EAB, which showed high efficiency in electron exchange between cells and the electrode (Gorby *et al.* 2006; Hau & Gralnick 2007). Recently, different biosensors based on *S. oneidensis* MR-1 have been developed (Si *et al.* 2016; Sun *et al.* 2017). This strain was proved to have high sensitivity to toxic stimuli, and also showed high efficiency to convert the cell stimuli to electric signal (Webster *et al.* 2014; Yang *et al.* 2018b). For example, with *S. oneidensis* MR-1 inoculated BES, a sensitive concentration response to the toxicity of formaldehyde was observed (Wang *et al.* 2013a). Moreover, acute toxicity of 3,5-dichlorophenol could also be assessed by a biosensor based on *S. oneidensis* MR-1 (Yang *et al.* 2018b). However, as most EAB are metal-reducing bacteria which could reduce the toxic heavy metal ions to low-/non-toxic forms, it is still unclear whether metal-reducing EAB can be used for heavy metal toxicity assessment or not.

In this study, a starvation pre-incubation procedure was established to avoid the heavy metal reduction by *S. oneidensis* MR-1 during the toxicity sensing process, which enabled accurate and sensitive toxicity assessment. Based on this starvation pre-incubation procedure, a mediator-free electrochemical biosensor for heavy metal toxicity assessment was developed and optimized. Compared with other heavy metal toxicity biosensors, by integrating the starvation pre-incubation, the mediator-free

biosensor developed here showed high sensitivity, good stability and reliability for real sample assessment, which implied that the EAB-based mediator-free biosensor holds great potential for water toxicity assessment.

## MATERIALS AND METHODS

### Bacteria strains and chemicals

*S. oneidensis* MR-1 (ATCC 700550) was cultivated in Luria-Bertani (LB) medium (peptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L, pH 7.0) with shaking at 30 °C under aerobic condition (Yong *et al.* 2014). After the cell density (OD<sub>600</sub>) reached the designed value (OD<sub>600</sub> of approximately 2), the bacteria were harvested by centrifugation and suspended in fresh electrolyte (M9 medium with Wolfe's minerals (Gorby *et al.* 2006)) with the designed cell density for biosensing assay (Si *et al.* 2015). *Escherichia coli* JM109 and *Pseudomonas aeruginosa* PAO1 (ATCC 15692) were cultured in LB liquid medium. The heavy metal solutions were prepared with various salts of CuSO<sub>4</sub>, CdCl<sub>2</sub>, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, and NiSO<sub>4</sub>. All chemical agents were analytical grade and purchased from Sangon Biotech (Shanghai, China).

### Electrochemical measurement

A three-electrode system composed of a 15-mL cylindrical borosilicate glass bottle containing a counter electrode (platinum wire), a reference electrode (saturated calomel electrode (SCE), +0.243 V vs. SHE), and a working electrode (WE) (1 cm × 2 cm carbon cloth) was constructed for electrochemical analysis. The electrochemical tests were performed under anaerobic condition. The amperometry was performed by a CHI-660E electrochemical workstation (Chenhua Instruments Co., Ltd, Shanghai, China). All potentials mentioned are reported versus SCE.

### Biosensing system assembly and toxicity assessment

For the biosensing system assembly, the cell suspension (suspended in electrolyte) was purged with N<sub>2</sub> for 30 min to remove the dissolved oxygen and then added into the three-electrode electrochemical cell. Later, the heavy metal stock solution (20 μL) was injected into the electrochemical cell to the designed final metal concentration.

Then, the electrochemical cells (cell + electrolyte + metal ions, without electron donor/carbon source) were incubated for 10–120 min, which was defined as the

pre-incubation period. The pre-incubation was performed at 30 °C with shaking (150 rpm) under anaerobic condition.

After the pre-incubation, lactate (purged with N<sub>2</sub>) was added as electron donor (10 mM) to start the bioassay. A poised potential was applied to the WE and the current output was monitored. For the toxicity assessment of real water, the samples were collected from the river at Jiangsu University (Jiangsu Province, China). All assays were conducted at 30 °C. All concentrations mentioned in this study are the final concentration in the electrochemical cell, if not stated otherwise. All tests are performed in triplicate. Statistical analysis was performed with one-way analysis of variance (ANOVA) using SPSS software.

The metal toxicity was represented by biosensor current output inhibition ratio. It was calculated with the following equation,

$$\text{Inhibition} = (i_0 - i_{in})/i_0 \times 100\%$$

where  $i_0$  indicates the current response of the biosensor without metal ions,  $i_{in}$  indicates the current response of the biosensor with metal ions.

The half maximal inhibitory concentration (IC<sub>50</sub>) of the biosensor towards different metal ion indicates the metal concentration that results in 50% inhibition ratio. The IC<sub>50</sub> for different metal ions was estimated with their calibration curves ( $Y = aX + b$ ,  $Y$  indicates the inhibition ratio (%),  $X$  indicates the concentration of heavy metal (mg L<sup>-1</sup>), respectively. So, the IC<sub>50</sub> was calculated as  $IC_{50} = (50 - b)/a$ .

## RESULTS AND DISCUSSION

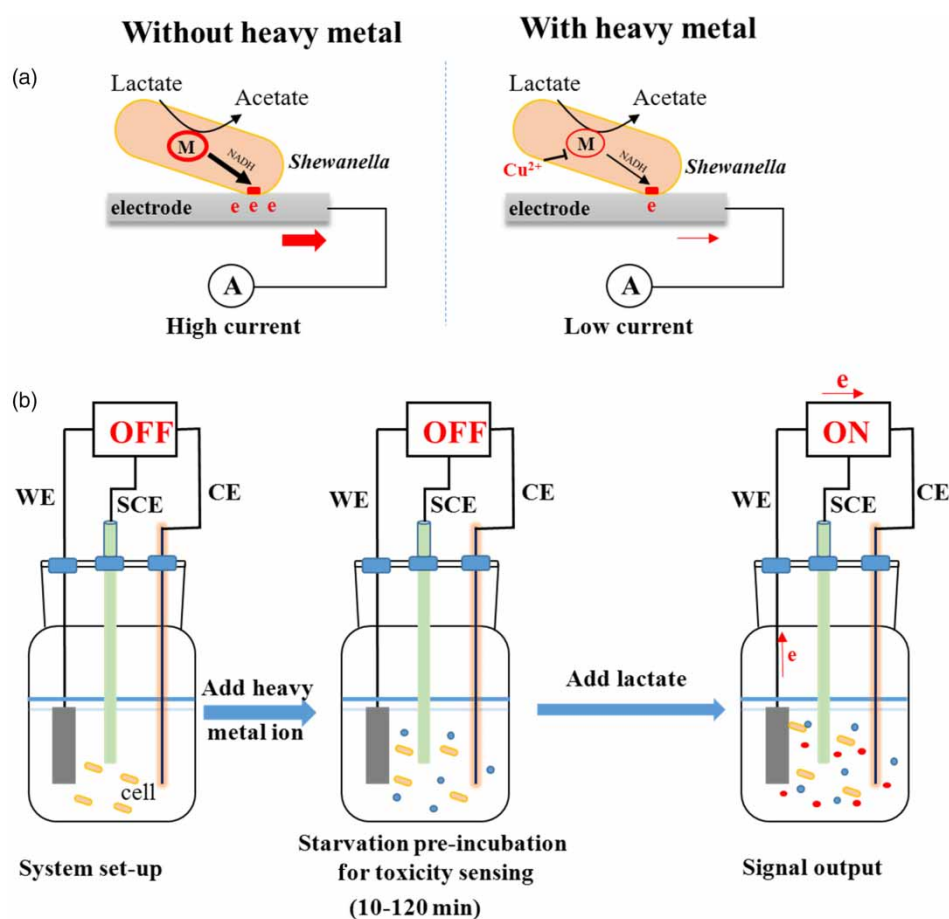
### Biosensing system development

*S. oneidensis* MR-1 is a model EAB that can transfer electrons derived from the cell metabolism to an electrode through a mediator-free manner with an efficient extracellular electron transfer pathway (Yong *et al.* 2014; Sun *et al.* 2017). Thus, it is reasonable to expect that the extracellular electron flow should be proportional to the activity of intracellular metabolism. It is well known that the activity of the cell metabolism is greatly affected by the environmental toxin, and the fluctuation of the metabolic activity has already been used as an indicator for toxicity assessment (Einicker-Lamas *et al.* 2002; Wang *et al.* 2013b). So, it is speculated that the heavy metal toxicity could be assessed by monitoring the response of extracellular electron flow

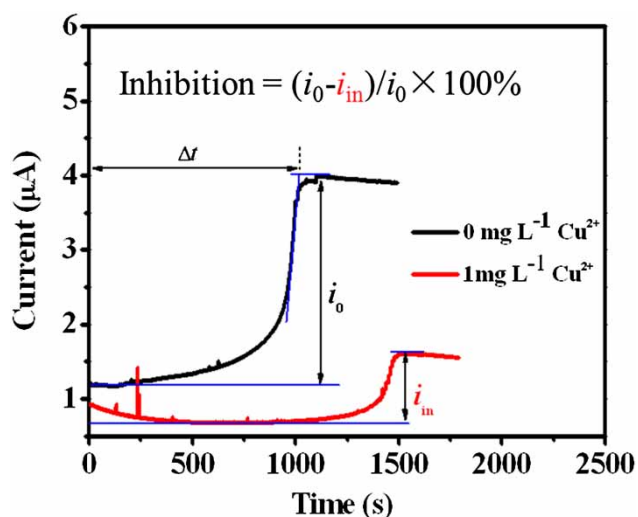
with a *S. oneidensis* MR-1-based mediator-free BES (Figure 1(a)). However, the feasibility of heavy metal ion detection is still doubtful because *S. oneidensis* MR-1 has strong reducing ability towards heavy metals such as Cr<sup>6+</sup> and Cu<sup>2+</sup> (Middleton *et al.* 2003; Kimber *et al.* 2018), which could result in a low toxic response (Figures S1 and S2, available with the online version of this paper).

To solve the problem of metal reduction, a starvation pre-incubation process was proposed. As the metal reduction was an electron donor driving process, it was found that the heavy metals could not be reduced under the starvation condition without electron donor (Figure S1). Thus, a starvation pre-incubation period might be useful for cells to sense the acute toxicity of the heavy metals by EAB. As compared with the non-starvation pre-incubation, the heavy metals with the same concentration showed much higher biotoxicity (Figure S2). Therefore, a biosensing procedure with a starvation pre-incubation period was established as the following: the *S. oneidensis* MR-1 cells were washed to remove the residual nutrient and resuspended in the M9 mineral medium without carbon source (electron donor). Then, heavy metal ion or water sample was added into the electron donor free cell suspension and incubated for 10–120 min (starvation pre-incubation for toxicity sensing). After this toxicity sensing period, electron donor was added into the system, and the bioelectrochemical activity of cells was evaluated by a three-electrode system (signal output step), which should be proportional to the toxicity of heavy metal (Figure 1(b)).

Furthermore, the feasibility for heavy metal toxicity assessment with the developed bioelectrochemical system was evaluated. In accordance with a previous report (Yang *et al.* 2018b), the *S. oneidensis* MR-1 cells generated a significant current output when electron donor (lactate) was injected and a poised potential was applied on the WE (Figure 2). After about 250 seconds' adaptation, the current output gradually increased for the cells without heavy metal addition. After that, a logarithmic increase was observed after about 800 seconds and reached a plateau of about 3.9 μA. In contrast, for cells with heavy metals addition, although the current evolution trend is similar to that without Cu<sup>2+</sup> addition, the adaptation time is much longer and the highest current output is much lower. As shown in Figure 2, with 1 mg L<sup>-1</sup> Cu<sup>2+</sup> addition, the time required to reach the highest current output ( $\Delta t$ ) is about 50% longer than that without Cu<sup>2+</sup> addition, while the highest current is only about 41% of that without Cu<sup>2+</sup> addition. Although the current at the start point of the biosensing process was unstable, a relatively stable maximum current



**Figure 1** | (a) Schematic of mediator-free *Shewanella*-based heavy metal toxicity assessment. (b) Operation procedure for starvation pre-incubation integrated heavy metal toxicity biosensing system. Carbon cloth is working electrode (WE), SCE is saturated calomel electrode, and platinum wire is the counter electrode (CE).



**Figure 2** | Amperometric response of *Shewanella*-based mediator-free biosensor to  $\text{Cu}^{2+}$ . The sensing conditions are as follows: starvation pre-incubation with  $\text{Cu}^{2+}$  for 30 min, 0.5 V poised potential on WE, and cell density at  $\text{OD}_{600} = 0.5$ .

output phase was observed after the logarithmic increase. Thus, the current increment could be considered as an indicator for toxicity assessment. The toxicity could be assessed with the inhibition ratio; 1 mg L<sup>-1</sup>  $\text{Cu}^{2+}$  resulted in an inhibition ratio of 32.7%, which is much more sensitive than other biosensing systems (Wang et al. 2013b; Gao et al. 2017). These results suggested that the toxicity of the heavy metal ions could be assessed by *S. oneidensis* MR-1 in a mediator-free manner with high sensitivity by integrating with a starvation pre-incubation process.

### System optimization for sensitivity improvement

The parameters such as poised potential, cell density, growth stage and pre-incubation time were optimized to further improve the performance of the biosensing system. Although the electric output of this biosensing system depends on the toxicity effect on the cell metabolism, the electron transportation efficiency between cells and the

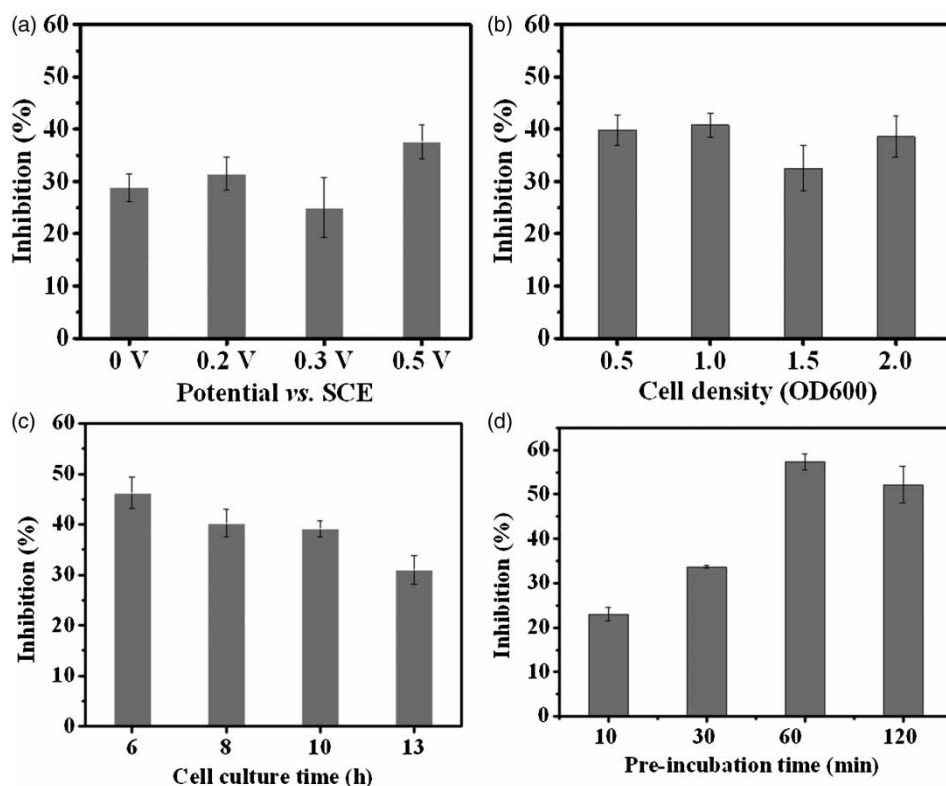
electrode also largely affects the output as it is the key linkage between cell metabolism and electric signal output on the electrode. Thus, the poised potential should be optimized. Various potential values from 0 V to 0.5 V (vs. SCE) were used and 0.5 V showed the highest inhibition ratio ( $37.6 \pm 3.2\%$ ) (Figure 3(a)). Further, the effect of cell density on the biosensor output was determined. It was found that the different cell densities did not show significant difference on the inhibition ratio among the tested cell densities. Thus, the lower cell density ( $OD_{600} = 0.5$ ) was selected as the optimum condition for easy operation. In addition, it was reported that the cells under different growth stage had very different sensitivity to the toxicity, while the cells at logarithmic growth phase were more sensitive (Wang *et al.* 2013b). As shown in Figure 3(c), compared with the cells at the very early logarithmic growth phase (6 h) (Figure S3, available online), prolonged cultivation (8, 10, and 13 h) gradually repressed the sensitivity as evidenced by the decreased inhibition ratio. Therefore, the cells at the early logarithmic growth phase

(6 h), which showed the highest inhibition ratio ( $46.2 \pm 3.1\%$ ), was selected as the optimum condition.

As starvation pre-incubation of cells with heavy metal ions is crucial for the toxicity assessment, the incubation time was further optimized. It was observed that the inhibition ratio increased with the prolonged pre-incubation time (Figure 3(d)), i.e., 10 min pre-incubation showed only  $23.0 \pm 1.5\%$  inhibition ratio, while 60 min pre-incubation reached the highest inhibition ratio of  $57.4 \pm 2.5\%$ . During the optimization, the inhibition ratio for  $0.5 \text{ mg L}^{-1} \text{ Cu}^{2+}$  was increased from  $28.8 \pm 2.7\%$  to  $57.4 \pm 2.5\%$ . Therefore, the optimum biosensing conditions were fixed as: 0.5 V poised potential on the WE, cell density of  $OD_{600} = 0.5$  at the early logarithmic growth phases (6 h), and the starvation pre-incubation time of 60 min.

### Analytical performance of the biosensing system

The analytical performance of this mediator-free biosensing system was determined under the optimized conditions.

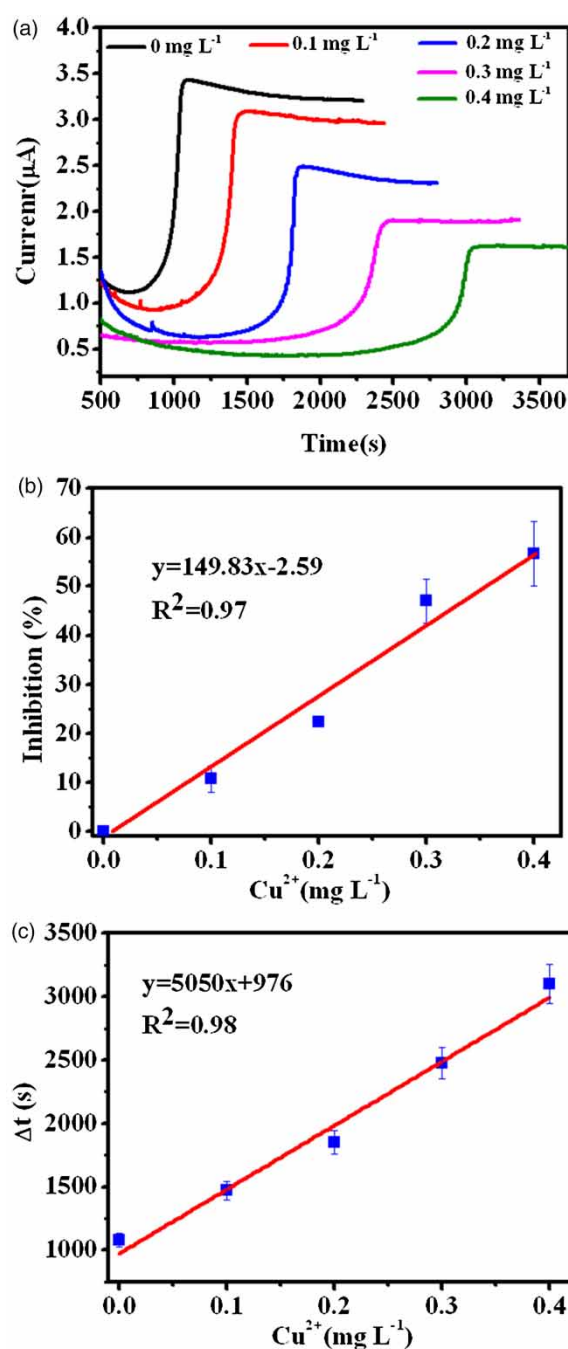


**Figure 3** | Effect of (a) poised potential (30 min pre-incubation, 0.5  $OD_{600}$  cell density, and 8 h cell culture time), (b) cell density (30 min pre-incubation, 8 h cell culture time, and 0.5 V WE potential), (c) cell culture time (30 min pre-incubation, 0.5  $OD_{600}$  cell density, and 0.5 V WE potential), and (d) pre-incubation time (0.5  $OD_{600}$  cell density, 0.5 V WE potential, and 6 h cell culture time) on the performance of biosensor ( $\text{Cu}^{2+}$ ,  $0.5 \text{ mg L}^{-1}$ ). The amperometric response without  $\text{Cu}^{2+}$  addition at designed condition was used as the control for inhibition ratio calculation.

The biosensor response to different concentrations of heavy metal was determined (Yang et al. 2017). As shown in Figure 4(a), the maximum current output was decreased with the increasing concentration of  $\text{Cu}^{2+}$ . In accordance with this, the inhibition ratio of  $\text{Cu}^{2+}$  was dependent on the concentrations. A good linear dependency ( $R^2 = 0.97$ ) between the inhibition ratio (%) and  $\text{Cu}^{2+}$  concentration ( $\text{mg L}^{-1}$ ) was observed as shown in Figure 4(b), suggesting that the proposed model of electrochemical biosensing system could be utilized for  $\text{Cu}^{2+}$  detection. The limit of detection (signal/noise = 3) calculated was  $0.03 \text{ mg L}^{-1}$ . Interestingly, higher concentration of  $\text{Cu}^{2+}$  resulted in a longer time ( $\Delta t$ ) to reach the maximum current output. Once  $0.4 \text{ mg L}^{-1} \text{ Cu}^{2+}$  was added,  $\Delta t$  was increased to about 3,000 seconds (about threefold higher than that without  $\text{Cu}^{2+}$  addition) (Figure 4(a)). More interestingly, it was found that the  $\Delta t$  also showed good linear dependency ( $R^2 = 0.98$ ) on the  $\text{Cu}^{2+}$  concentrations, suggesting  $\Delta t$  would be another indicator for heavy metal ion detection (Figure 4(c)). Furthermore, the concentration response of this biosensing system to other heavy metals ( $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ , or  $\text{Cr}^{6+}$ ) was also determined (Table S1, available online) and good concentration dependency was achieved, respectively. The results suggested that the system and method developed here might be applicable for heavy metal toxicity assessment in water systems.

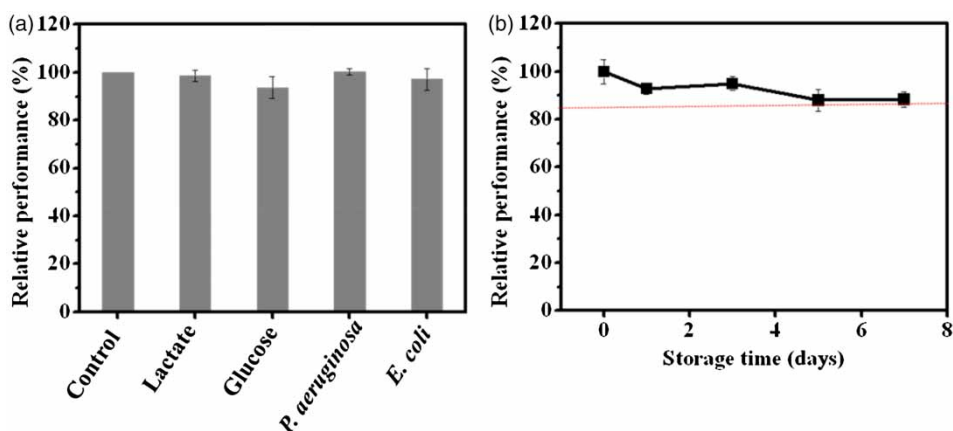
The real water samples might contain various organic compounds or bacteria which might affect the assessment. Thus, possible interferences from organic compounds (lactate was selected as it is the most common electron donor for *S. oneidensis* MR-1, glucose was selected as it is the most common organic compound in the environment) and bacteria (*E. coli* and *P. aeruginosa* were selected due to their prevalence in the environment) were tested. As shown in Figure 5(a), no significant performance difference could be observed among the biosensors with or without lactate or glucose addition. In addition, simulated bacteria contamination from *P. aeruginosa* or *E. coli* did not show significant influence on the biosensing output. The results suggested that the *Shewanella*-based mediator-free biosensing system was robust enough for water toxicity assessment.

Storage stability (shelf life-time) is another concern for practical application of whole-cell biosensors, since long-term storage of microorganism at low temperature may result in decreased cell viability (Si et al. 2015). However, *S. oneidensis* MR-1 is a low temperature resistant strain (Abboud et al. 2005; Si et al. 2015). It was found that storage at  $4^\circ\text{C}$  showed only a marginal effect on the analytic



**Figure 4** | (a) Dose-dependent response of *Shewanella*-based mediator-free biosensor to  $\text{Cu}^{2+}$  under optimized conditions. (b) Linear relationship between inhibition ratio and  $\text{Cu}^{2+}$  concentrations assessed by the developed biosensor. (c) Linear relationship between  $\Delta t$  and  $\text{Cu}^{2+}$  concentrations assessed by the developed biosensor.

performance, i.e., after 7 days' storage at  $4^\circ\text{C}$ , only ~12% of biosensing activity decrease was observed. The good long-term storage stability implied that this biosensor could be further adapted for practical application.



**Figure 5** | (a) Interference resistance of the developed biosensor.  $\text{Cu}^{2+}$  added is  $0.3 \text{ mg L}^{-1}$ ; The inhibition ratio of  $\text{Cu}^{2+}$  without interference (control) was normalized as 100%, while the relative performance was calculated as (inhibition ratio with interference/inhibition ratio of control)  $\times 100\%$ . The concentrations of the interferences are as the following: glucose  $10 \text{ mM}$ , lactate  $10 \text{ mM}$ , *P. aeruginosa*  $10^7 \text{ CFU mL}^{-1}$ , and *E. coli*  $10^7 \text{ CFU mL}^{-1}$ . Statistical analysis was performed using one-way ANOVA ( $P > 0.05$ ). (b) Storage stability ( $4^\circ \text{C}$ ) of the developed biosensor. The inhibition ratio towards  $0.3 \text{ mg L}^{-1} \text{ Cu}^{2+}$  before storage was normalized as 100%.

### Assessment of toxicity from different heavy metal ions in water

The biosensing performance with simulated real samples of river water was also determined. It was found that the biosensing method developed here showed a high reliability (recovery efficiency  $> 86\%$ ) and good reproducibility (the coefficient of variation  $< 6\%$ ) (Table S2, available online), which suggested its potential for practical application.

Moreover, the  $\text{IC}_{50}$  for different heavy metal ions was determined (Table 1). The  $\text{IC}_{50}$  values obtained were 0.35, 2.48, 3.49, and  $6.52 \text{ mg L}^{-1}$  for  $\text{Cu}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Cd}^{2+}$ , respectively. The toxicity of these heavy metals were determined as  $\text{Cu}^{2+} > \text{Cr}^{6+} > \text{Ni}^{2+} > \text{Cd}^{2+}$ . The results are in good agreement with other reports and indicated that the

copper ion is more toxic than other ions to microorganisms (Gao *et al.* 2017).

The amperometric method is a sensitive and commonly used technique for assessment of the biotoxicity of heavy metal ions. However, previously used bacteria such as *E. coli* and *Psychrobacter* sp. were non-EAB, which required exogenously added mediators to transport the electrons between cells and the electrode to link the cell metabolism with electric output (Wang *et al.* 2013b). As *S. oneidensis* MR-1 is an EAB which could transport the electrons between cells and the electrode without mediator, the biosensing system developed here showed the unique advantage of being mediator-free, which might reduce the cost, simplify the operation, and also avoid possible toxicity from the exogenously added mediators.

**Table 1** | Performance comparison of various whole-cell biosensors for heavy metal ions toxicity assessment

Method	Microorganism	$\text{IC}_{50}$ ( $\text{mg L}^{-1}$ )				Reference
		$\text{Cu}^{2+}$	$\text{Ni}^{2+}$	$\text{Cd}^{2+}$	$\text{Cr}^{6+}$	
Amperometry, mediator-less	<i>Shewanella oneidensis</i>	0.35	3.49	6.52	2.48	This study
Amperometry, ferricyanide mediator	<i>Escherichia coli</i>	3.71	– <sup>a</sup>	7.8	–	Catterall <i>et al.</i> (2010)
Amperometry, <i>p</i> -benzoquinone mediator	Mixed microbial consortium	16.5	–	20.5	–	Gao <i>et al.</i> (2016)
Amperometry, <i>p</i> -benzoquinone mediator	<i>Psychrobacter</i> sp.	2.6	–	47.3	14.0	Wang <i>et al.</i> (2013b)
Amperometry, ferricyanide–menadione double-mediator	<i>Saccharomyces cerevisiae</i>	10.12	17.06	13.88	–	Gao <i>et al.</i> (2017)
Bioluminescence	Engineered <i>Acinetobacter baylyi</i>	0.68	–	43.67	–	Cui <i>et al.</i> (2018)
Colorimetry	<i>Vibrio fischeri</i>	41.5	–	33.1	35.6	Dalzell <i>et al.</i> (2002)

<sup>a</sup>Not mentioned.

More impressively, the IC<sub>50</sub> values obtained by this bio-sensing system were much lower than previously reported mediator-based biosensing systems or the most conventional bioluminescence sensing systems. For example, it was found that non-EAB *E. coli* and *Psychrobacter* sp. needed extra mediators to facilitate electron transportation and showed high IC<sub>50</sub> values for Cu<sup>2+</sup> detection (Catterall *et al.* 2010; Wang *et al.* 2013b), and the mixed microbes showed the highest IC<sub>50</sub>, up to 16.5 mg L<sup>-1</sup> (Table 1). In contrast, the *S. oneidensis* MR-1 showed the lowest IC<sub>50</sub> value of 0.35 mg L<sup>-1</sup> without exogenously added mediator. The bioluminescence method also showed an IC<sub>50</sub> twice that of the method developed here (Zhu *et al.* 2017; Cui *et al.* 2018). The results indicated that the mediator-free biosensing system developed here holds great potential for a simple, cost-effective, and sensitive assessment of the water toxicity from heavy metals and should be a promising tool for early warning of the water toxicity.

## CONCLUSIONS

In this work, a mediator-free BES for sensitive assessment of heavy metal toxicity was developed by using *S. oneidensis* MR-1 integrated with starvation pre-incubation. The procedure established here successfully excluded the reduction of the heavy metal ions during the toxicity sensing period, which in turn enabled a higher accuracy and sensitivity. Moreover, the biosensing conditions including poised potential, cell density, growth stage and pre-incubation time were optimized. The IC<sub>50</sub> values obtained with this method were much lower than the other reported bioelectrochemical methods. In addition, as *S. oneidensis* MR-1 is an excellent EAB, this biosensing system showed the unique advantage of being a mediator-free operation. To the best of our knowledge, this is the first pure culture mediator-free BES for heavy metal toxicity assessment. Thus, this work provided a simple, cost-effective, sensitive and robust tool for heavy metal toxicity assessment in water systems, and implies that it is feasible to use a pure EAB strain for heavy metal biosensor (especially for specific recognition with genetic engineering) development, which may broaden the toolbox for water quality monitoring.

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