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RESEARCH PAPER

Enhancing the sensitivity of water toxicity detection based on suspended *Shewanella oneidensis* MR-1 by reversing extracellular electron transfer direction

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Abstract

Water toxicity detection is of great significance to ensure the safety of water supply. With suspended electrochemically active bacteria (EAB) as the sensing element, a novel microbial electrochemical sensor (MES) has recently been reported for the real-time detection of water toxicity, but its practical applications need to further improve the sensitivity. Extracellular electron transfer (EET) is an important factor affecting MES performance. In the study, the EET of suspended EAB–based MES was optimized to further enhance the sensitivity. Firstly, by using a model EAB stain *Shewanella oneidensis* MR-1, it was revealed that the sensitivity was increased at most 2.7 times with inward EET (i.e., cathodic polarization). Then, a novel conjecture based on electron transfer and energy fluxes was proposed and testified to explain this phenomenon. Finally, three key operating parameters of inward EET were orthogonally optimized. The optimized parameters of inward EET included a potential of − 0.5 V, a cell density of 1.8×10^8 CFU/mL, and an electron acceptor concentration of 15 mM.

Keywords Water toxicity detection · Bidirectional extracellular electron transfer · Microbial electrochemical sensor · Sensitivity · Electrochemically active bacteria

Introduction

Acute water pollution emerges largely worldwide with the rapid development of industry and agriculture. According to the announcement issued by the United Nations, 90% of the surface water is polluted, and 1/3 of water supply safety is threatened, resulting in the death of more than 600 children every day [1, 2]. The monitoring of water quality is the key to realize the early warning of water pollution. The traditional methods for water quality monitoring are mainly based on physical or chemical detection technologies, which are capable of accurately quantifying the toxic pollutants in water [3]. However, these methods rely on large and expensive equipment, which fail to reveal the biotoxicity and comprehensive toxicity of water. An alternative way that employs bio-elements (e.g., tissue, cell, or protein) as the indicators directly detects water toxicity, which is based on biological responses to environmental changes [4]. A series of indicators, including fish, algae, and bacteria, were reported to realize water toxicity monitoring [5]. Among these indicators, electrochemically active bacteria (EAB) with the ability of extracellular electron transfer (EET) attracted lots of attention during recent years [6]. The metabolism activity of EAB is reflected by the EET rate (i.e., current), and the current is depressed once EAB are exposed to toxic pollutants. Based on the principle, water toxicity detection is realized by measuring currents directly [7]. Compared with other bioassays, EAB are capable of detecting water toxicity without external signal transducers

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and the detection is independent of water color and salinity of water, showing great prospects in the field of water quality monitoring [8].

Numerous studies have proved the feasibility of EAB–based water toxicity monitoring, and a series of toxic pollutants have been successfully detected [9–12]. In these studies, EAB biofilms were used as the sensing elements, which realized self-renewal and self-maintenance [13]. However, the complex components in the biofilm, such as protein, polysaccharide, and peptidoglycan, act as a natural barrier of EAB within the biofilm, which reduces the sensitivity of toxicity detection [14]. A novel water toxicity detection method based on suspended EAB was proposed to solve this problem, which successfully detected low-concentration pollutants without forming biofilm [12, 15]. Additionally, the enhancement of sensitivity by replacing EAB biofilms with suspended EAB was first quantified, and the results showed that the sensitivity of suspended EAB in water toxicity detection was 30 times higher than that of the biofilms [16]. Nonetheless, the reported detection limit of pollutants based on suspended EAB still exceeded standard values with the tightening of the water quality standard [17]. Therefore, more efforts are eagerly needed to enhance the sensitivity of suspended EAB–based water monitoring for practical applications.

EET is related to water toxicity and EAB metabolism, and regulating EET may be an efficient way to enhance sensitivity. Based on electron transfer direction, EET includes outward EET and inward EET. With outward EET, electrons are transferred from the respiratory chain to an electrode, and currents are generated with continuous electron flow. When outward EET is reversed, electrons are transferred from an electrode to the respiratory chain and terminal electron acceptors (e.g., nitrate, fumarate, and TMAO), and this process is inward EET [18]. Most previous studies utilized the outward EET and output current of EAB as the electrical signals to monitor water quality [19]. Jiang et al. firstly employed the inward EET and input current of EAB to detect water toxicity, which aimed to avoid the change in organic matters masking the effects of toxic pollutants on the electrical signals of outward EET. Compared with outward EET, EAB with inward EET exhibited a higher water toxicity detection sensitivity, and achieved the rapid detection of 0.0005% formaldehyde [20]. This phenomenon was further confirmed by the following studies. The sensitivity with inward EET was 1–9 times higher than that with outward EET by detecting Hg^{2+}, Cr^{6+}, and Pb^{2+} [21, 22]. However, although inward EET seemed to improve water toxicity detection, the underlying mechanism of improved sensitivity was still unclear [23]. The main reason is that these studies all used mixed-culture EAB biofilms, and the reversal of the EET direction may change EAB community structure and biofilm property simultaneously [24], which were proved to be the key parameters affecting water toxicity detection. Therefore, the direct effects of EET direction on water toxicity detection remained to be revealed.

A recent study has reported a novel microbial electrochemical sensor (MES) based on suspended *Shewanella oneidensis* MR-1 [25], and the bidirectional EET capacity of *S. oneidensis* MR-1 has been proved [12, 26]. In the study, MESs based on pure cultured and suspended *S. oneidensis* MR-1 were used to further investigate the direct effects of EET direction on water toxicity detection. Firstly, MESs with inward and outward EET were constructed. Then, two common pollutants were tested under different EET conditions, and the differences in the sensitivity were demonstrated. After that, the electrochemical responses of outward and inward EET to a toxic shock were compared with the identical sensing element. Based on these results, the underlying mechanism of differences in the sensitivity under different EET direction conditions was revealed. Finally, three key parameters affecting the current of suspended *S. oneidensis* MR-1 were orthogonally optimized by using the optimized EET direction.

Materials and methods

Microbial cultivation

S. oneidensis MR-1 (ATCC 700,550) was obtained from ATCC and refrigerated at − 80 °C. Before use, *S. oneidensis* MR-1 was activated in Luria–Bertani medium overnight, and the aerobic incubation conditions were set as follows: 0.5% of inoculum, 22 °C of culture temperature, and 180 r/min of shaker rotational speed. After cultivation, *S. oneidensis* MR-1 suspension (OD_{600} = 2.0) was reserved at 4 °C to construct MESs.

MES construction

Eight MESs (MESs 1–8) were constructed according to our previous study [27]. All MESs were identical, and each MES was composed of a piece of 2 cm × 2 cm carbon cloth as the working electrode, 1 cm × 1 cm Pt slide as the counter electrode, and Ag/AgCl (3 M KCl) electrode as the reference electrode. The carbon cloth was immersed in acetone and heated at a high temperature before use. All components were cleaned thoroughly with double-distilled water and sterilized before use. The sterilization referred to a previous study to avoid potential damages to Ag/AgCl electrodes.

After preparation, all the MESs were divided into three groups, including outward MESs (MESs 1–3), inward MESs (MESs 4–6), and abiotic control (MESs 7–8). Both outward MESs and inward MESs (MESs 1–6) used the same electrolyte, which aimed to reveal the direct effects of EET

direction on water toxicity detection. The electrolyte in MESs 1–6 included 20 mL of *S. oneidensis* MR-1 suspension and 20 mL of defined medium (DM). Twenty milliliters of sterile Luria–Bertani medium and 20 mL of DM medium were added to MESs 7–8. Each liter of DM contained 1 g of $NaHCO_3$, 0.13 g of KCl, 0.027 g of $CaCl_2$ $2H_2O$, 0.2 g of $MgCl_2$ $6H_2O$, 5.85 g of NaCl, 7.2 g of HEPES, and 10 mM of trimethylamine N-oxide (TMAO). TMAO is the electron acceptor of *S. oneidensis* MR-1 [28]. After that, outward MESs (MESs 1–3) were used to develop the outward EET of *S. oneidensis* MR-1 with a constant potential of 0.5 V, and inward MESs (MESs 4–6) were used to develop the inward EET of *S. oneidensis* MR-1 with a constant potential of − 0.5 V. MESs 7–8 were used as abiotic control, and the working potentials of MESs 7–8 were set at 0.5 V and − 0.5 V, respectively. Finally, all currents of MESs were measured continuously, and the MESs were ready for toxic tests once the currents became stable.

Toxic tests

The spike tests were used to simulate acute toxic shocks. Each toxic test was conducted in the following three steps. First, MESs with suspended *S. oneidensis* MR-1 as the sensing elements were constructed, and the currents of MESs were measured. Then, a specific volume of pollutant concentrate was spiked into MESs when the currents became stable. The toxic exposure lasted for 30 min, and the currents were recorded continuously. Finally, the MESs were cleaned up, and a new piece of carbon cloth was used for the next toxic test. The response of *S. oneidensis* MR-1 to the toxic shock was calculated based on the inhibition ratio of the current, which was determined by using the following equation,

$$\mathrm{IR} = \left(I_1 - I_2\right)/I_1 \times 100\% \quad (1)$$

where I1 and I2 were the current values before and after the toxic exposure.

Bacterial viability analysis

Confocal laser scanning microscopy (CLSM) was used to analyze the viability of *S. oneidensis* MR-1. Before CLSM analysis, 100 µL of *S. oneidensis* MR-1 suspension was sampled from non-toxic inward MESs and outward MESs, respectively. After that, all MESs were shocked with 0.05 mg/L of Cd^{2+} for 30 min, and then the sampling was performed again. All samples were stained with a LIVE/DEAD BacLight Bacterial Viability Kit (7012, Invitrogen, USA) according to the manufacturer's protocols and were observed with CLSM (TCS SP8, Leica, Germany). At least three locations were randomly selected for each sample. Viability was defined as the proportion of alive *S. oneidensis* MR-1, and it was analyzed based on the following equation,

$$\mathrm{Viability} = Int_{\mathrm{L}}/(Int_{\mathrm{L}} + Int_{\mathrm{D}}) \times 100\% \quad (2)$$

where Int_{L} and Int_{D} referred to the optical intensity of alive and dead *S. oneidensis* MR-1, respectively.

Electrochemical measurements

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to reveal the underlying mechanism of differences in the sensitivity between inward and outward MESs. Two MESs (randomly chosen from MESs 1–6) were refreshed and used to test the toxic shock of 0.05 mg/L Cd^{2+}. As the MESs were capable of performing bidirectional EET, the electrochemical responses of outward and inward EET to a toxic shock were compared with the identical sensing element. CV was measured using a multi-channel potentiostat (CHI1030C, ChenHua, China), and the detailed measurement parameters were set as follows: the potential range of − 0.5 to 0.5 V and the scan rate of 1 mV/s. The currents with the potentials of 0.5 V and − 0.5 V referred to anodic limiting current and cathodic limiting current in the CV curves, which represented the electrochemical activity of anodic polarization and cathodic polarization, respectively [29]. EIS was obtained with an electrochemical station (Zennium E, Zahner, Germany) and measured with the following parameters: the frequency range of 50 mHz to 100 kHz and a small disturbance voltage of 5 mV. The obtained EIS data were analyzed with a classical electrochemical model by using Zman, which was briefly described by $R_s - (R_{ct} - W)|\mathrm{CPE}$. In this model, R_{ct} is charge transfer resistance, which represents the electrochemical activity of *S. onediensis* MR-1, and R_s, W, and CPE represent ohmic resistance, diffusion resistance, and double layer capacitance, respectively.

Orthogonal optimization

Three key parameters, including cell density (representing catalyst concentration), electrode potential (representing electron donor concentration), and TMAO concentration (representing electron acceptor concentration), were optimized to improve the sensitivity of water toxicity detection based on suspended *S. oneidensis* MR-1 with inward EET. The orthogonal design was used to simplify experiments. Three levels of each parameter were selected, and a total of 9 experiments was designed based on normalized orthogonal table L9 (3^4). The detailed levels of each parameter are shown in Table 1. The range analysis was used to determine the sensitivity of factors to the experimental results according to the previous study.

Table 1 Orthogonal experimental factors table

Factors	Level 1	Level 2	Level 3
Cell density (CFU/mL)	1.8×10^8	2.4×10^8	3×10^8
Electrode potential (V)	−0.4	−0.45	−0.5
TMAO concentration (mM)	5	10	15

Results and discussion

Current generation of *S. oneidensis* MR-1 with outward and inward EET

The previous studies demonstrated that suspended *S. oneidensis* MR-1 is capable of outward EET and inward EET, and *S. oneidensis* MR-1 performed outward EET and inward EET at different electrode potentials, respectively [30]. As shown in Fig. 1a, there were obvious output currents at a constant potential of 0.5 V (MESs 1–3), indicating that *S. oneidensis* MR-1 respired with organic matters as the electron donor and an electrode as the electron acceptor [16]. There was obvious current consumption when the electrode potential was set as − 0.5 V (MESs 4–6), which showed that *S. oneidensis* MR-1 respired with an electrode as the electron donor and TMAO as the electron acceptor (Fig. 1b). Additionally, both the outward and inward currents remained stable after the capacitive currents disappeared, which indicated that both outward EET and inward EET of *S. oneidensis* MR-1 were feasible for toxicity detection.

Notably, the baseline value of the inward current (− 105.7 μA) was obviously higher than that of the outward current (45.9 μA), and a similar phenomenon was also observed by using other EAB strains capable of bidirectional EET, which might be attributed to the fact that inward EET was more conducive to the completion of transmembrane electron transfer [31, 32]. The speculation was not suitable to explain the obtained results in this study, because *S. oneidensis* MR-1 was proved to utilize the same EET pathway to perform outward EET and inward EET [33]. In fact, considering the energy fluxes under different EET directions, outward EET only represented a small amount of electron transfer in the respiratory chain [34], while inward EET included all the electron transfer of the respiratory chain [35]. Therefore, an alternative explanation based on energy fluxes was more suitable; inward EET completely reflected bacterial energy metabolism, which resulted in the higher inward currents (Fig. 2). In addition, the working potentials were also different under the two EET conditions, and the higher currents might be due to the higher overpotential of inward EET. More efforts are still needed to further explain the higher currents of inward EET in future studies.

Water toxicity detection by using *S. oneidensis* MR-1 with outward and inward EET

Two types of MESs (outward MESs and inward MESs) were used to conduct toxic tests. The toxic pollutants tested included heavy metal (Cd^{2+}) and organic pollutants

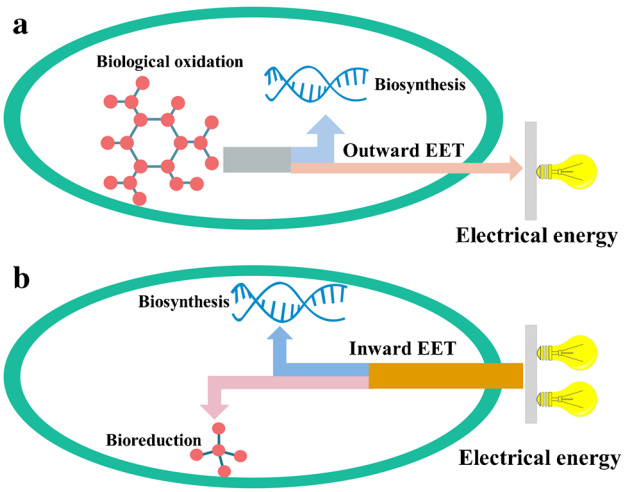

Fig. 2 Schematic diagram of different energy fluxes in outward EET (**a**) and inward EET (**b**) conditions. Outward EET only represents a small amount of the electron transfer of the respiratory chain, while inward EET includes all the electron transfer of the respiratory chain

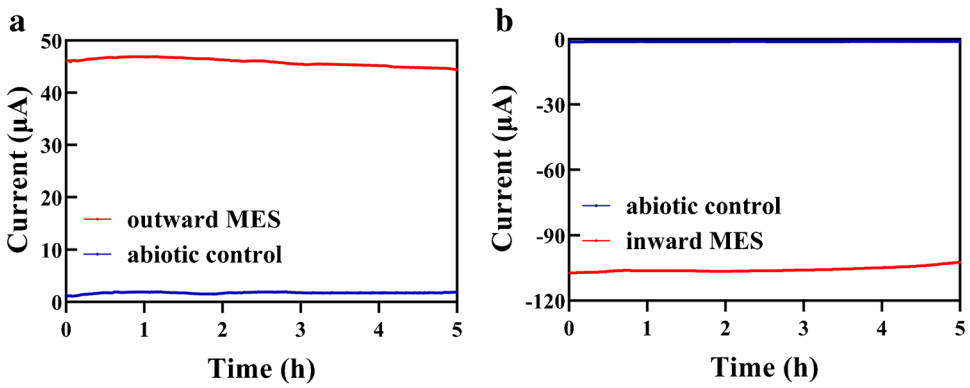

Fig. 1 The *i-t* curves of *S. oneidensis* MR-1. **a** Current generation of an outward MES and abiotic control at the potential of 0.5 V. **b** Current consumption of an inward MES and abiotic control at the potential of − 0.5 V

(phenol), and the tested concentrations ranged from 0.05 to 0.5 mg/L. As shown in Fig. 3a, b, both currents of outward MESs and inward MESs decreased sharply with the addition of 0.05 mg/L Cd^{2+}, which indicated the strong toxicity of Cd^{2+} for *S. oneidensis* MR-1 [36]. Compared with outward MESs, inward MESs exhibited a higher sensitivity. Specifically, the IR of inward MESs was 13.2% ± 1.8% under the same toxic shock of 0.05 mg/L Cd^{2+}, while that of outward MESs was only 5.6% ± 1.4%. A similar phenomenon was also observed in the high-concentration Cd^{2+} detection; the IR with inward MESs was 19.8% ± 1.0%, 24.7% ± 1.4%, and 35.4% ± 1.3%, while that with outward MESs was 10.7% ± 0.8%, 18.6% ± 0.6%, and 28.7% ± 2%. In addition, the IR of inward MESs was at most 2.7 times higher than that of outward MESs for the detection of phenol, which further confirmed that inward MESs were more sensitive to the detection of toxic pollutants. Interestingly, the enhancement of toxicity detection sensitivity using inward EET instead of outward EET also varied with the concentration of pollutants. The enhancement of sensitivity became more evident with the decrease of pollutant concentration. For example, the sensitivity was increased by 2.3 times when detecting 0.05 mg/L Cd^{2+}, while that was only increased 1.2 times for 0.5 mg/L Cd^{2+} detection, which indicated a unique advantage of *S. oneidensis* MR-1 with inward EET for detecting trace pollutants.

Viability analysis of *S. oneidensis* MR-1

The sensitivity differences between outward MESs and inward MESs were investigated by using variance analysis. The IR of inward MESs was significantly higher than that of outward MESs under all tested shocks, suggesting that *S. oneidensis* MR-1 with inward EET was more sensitive to water toxicity detection. Two possible reasons were proposed to explain the phenomenon. The first one was that inward MESs may exhibit a lower toxic resistance, and the higher sensitivity was probably due to the inactivation of more *S. oneidensis* MR-1 cells after toxic shocks under inward EET condition. The CLSM images of bacterial metabolism activity before and after 0.05 mg/L Cd^{2+} toxic shock were obtained to verify this speculation, and the viability changes after the toxic shock were calculated [16]. As shown in Fig. 4a and c, it was observed that *S. oneidensis* MR-1 with inward and outward EET possessed high viability before the toxic shock, reaching 95% under different EET conditions. After 30 min of 0.05 mg/L Cd^{2+} exposure, the viability of *S. oneidensis* MR-1 with both inward and outward EET decreased obviously,

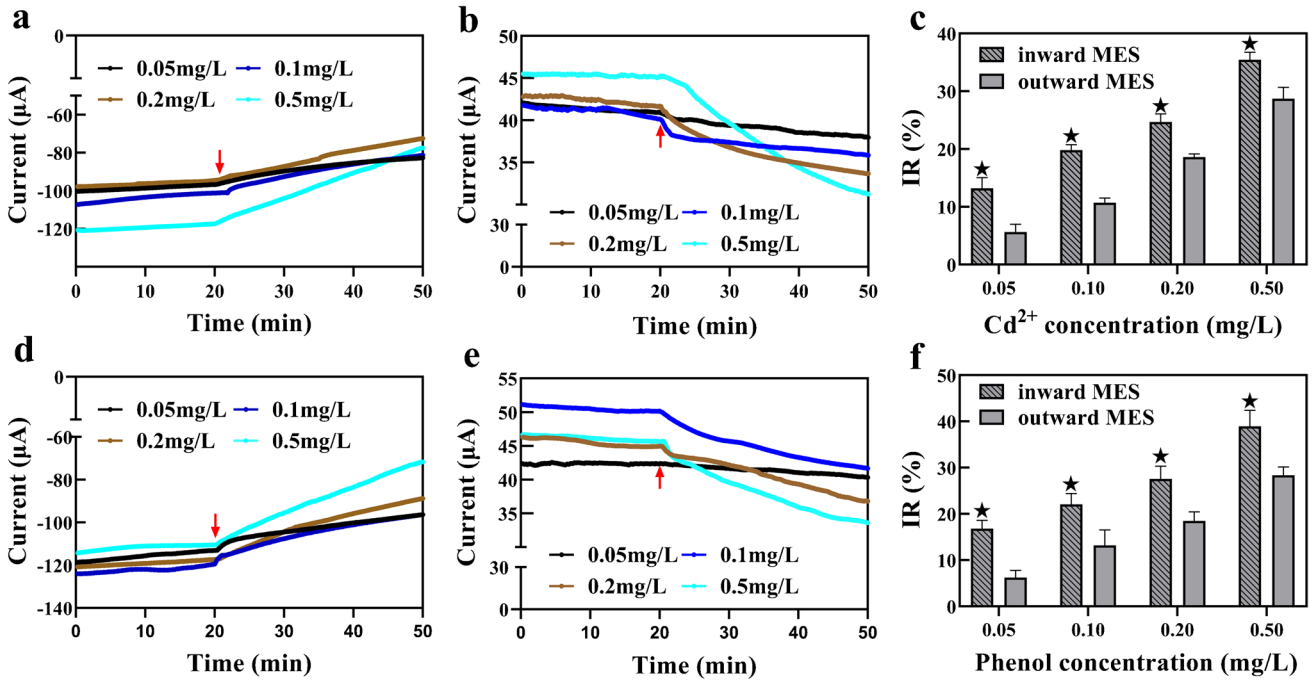

Fig. 3 Toxic shock tests with different MESs. **a** Current responses of inward MESs to a series of Cd^{2+} shocks. **b** Current responses of outward MESs to a series of Cd^{2+} shocks. The tested Cd^{2+} concentrations ranged from 0.05 to 0.5 mg/L, and the exposure time was 30 min. **c** IR value comparison of inward and outward MESs at different Cd^{2+} concentration conditions. **d** Current responses of inward MESs to a series of phenol shocks. **e** Current responses of outward MESs to a series of phenol shocks. **f** IR value comparison of inward and outward MESs at different phenol concentration conditions. ⋆ represent significant differences in IR values between inward and outward MESs ($P < 0.05$)

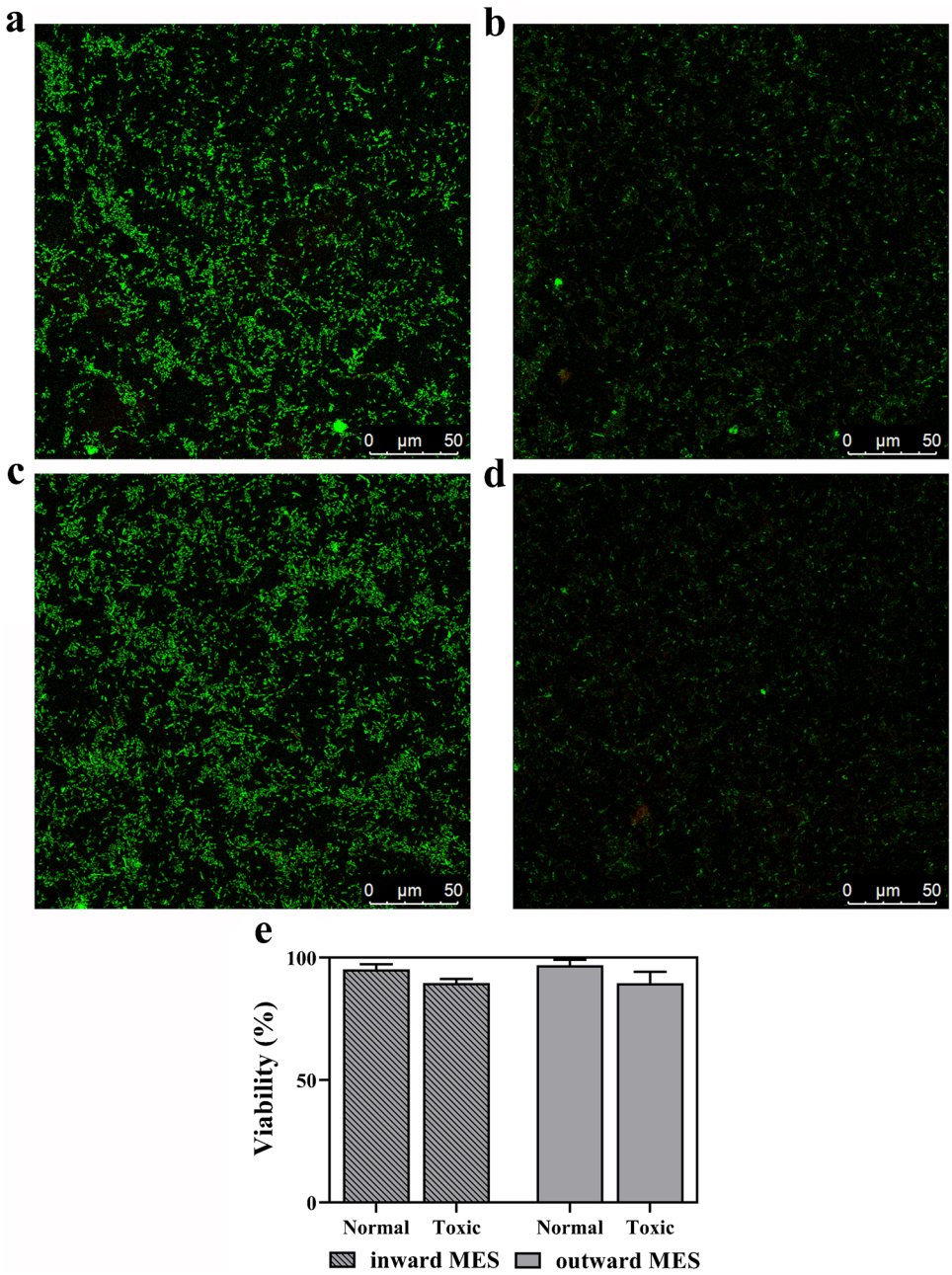

Fig. 4 CLSM images and viability analysis. **a** CLSM image of *S. oneidensis* MR-1 suspension in an inward MES before the shock of 0.05 mg/L Cd^{2+}. **b** CLSM image of *S. oneidensis* MR-1 suspension in an inward MES after 30 min of 0.05 mg/L Cd^{2+} exposure. **c** CLSM image of *S. oneidensis* MR-1 suspension in an outward MES before the shock of 0.05 mg/L Cd^{2+}. **d** CLSM image of *S. oneidensis* MR-1 in an inward MES after 30 min of 0.05 mg/L Cd^{2+} exposure. Live cells were imaged as green, whereas dead cells were imaged as red. **e** Cell viability before and after the toxic shock

demonstrating the high bio-toxicity of Cd^{2+} [36]. However, it was interesting to reveal that the decline of viability was basically the same under different EET conditions. Specifically, the viability of *S. oneidensis* MR-1 decreased from 95.18% ± 2.14% to 89.66% ± 1.64% under the inward EET condition, while that decreased from 96.85% ± 2.27% to 89.51% ± 4.68% under the outward EET condition. Therefore, the first speculation cannot explain the observation that the sensitivity was enhanced by using inward EET, and there was another reason for the higher sensitivity of *S. oneidensis* MR-1 under the inward EET condition.

Another reason may be that the slight damages of cell metabolism cannot induce the reduction of outward EET current directly. The previous studies also reported that the output currents remained unchanged even if the metabolic activity of EAB was significantly inactivated [22, 37]. These phenomena demonstrated that the output currents of EAB failed to reflect bacterial activity accurately, which was probably because outward EET was only part of the electron transfer in the respiratory chain. Differently, inward EET included all the electron transfer of the respiratory chain, and it was reasonable that the slight inhibition of cell

metabolism would decrease the input current directly. The results of toxic tests partly confirmed this conjecture, and the sensitivity enhancement with inward EET was more obvious when detecting low-concentration pollutants. Therefore, it was assumed that different energy fluxes under the two EET conditions were the main reason for the higher sensitivity with inward EET, which remained to be testified in the following studies.

Mechanism of the enhanced sensitivity with inward EET

CV curves of MESs capable of bidirectional EET before and after 30 min of 0.05 mg/L Cd^{2+} exposure were obtained to further testify the conjecture (Fig. 5a–c). Before the toxic test, CV curves exhibited obvious anodic and cathodic polarization with the applied potential of 0.5 V and − 0.5 V, which confirmed the bidirectional EET capacity of suspended *S. oneidensis* MR-1 [38]. The cathodic polarization decreased significantly after the toxic exposure, and the limiting current of cathodic polarization decreased 17.0% ± 4.5% (from − 147.3 to − 117.6 μA). However, the anodic polarization remained basically unchanged after the toxic exposure, and the limiting current of anodic polarization only slightly decreased 4.1% ± 1.0% (from 120.5 to 116.4 μA). The anodic and cathodic polarization reflected the electrochemical activity of outward EET and inward EET, respectively [39], and the results indicated that inward EET and input currents of EAB were more sensitive to toxic shocks. EIS data further confirmed this conclusion. As shown in Fig. 5e–g, the R_{ct} of cathodic polarization increased 41.7% (from 586.3 Ω ± 3.5 Ω to 830.7 Ω ± 5.4 Ω) with a MES capable of bidirectional EET, while that of anodic polarization only increased 12.3%. Therefore, inward EET of EAB more efficiently reflected the changes of cell metabolism, resulting in the higher sensitivity for water toxicity detection in this study.

The previous studies also reported that mixed cultured EAB exhibited higher sensitivity for water toxicity detection after reversing outward EET to inward EET [19]. However, EAB community structure and biofilm properties vary with the direction of EET, and the mechanism of enhanced

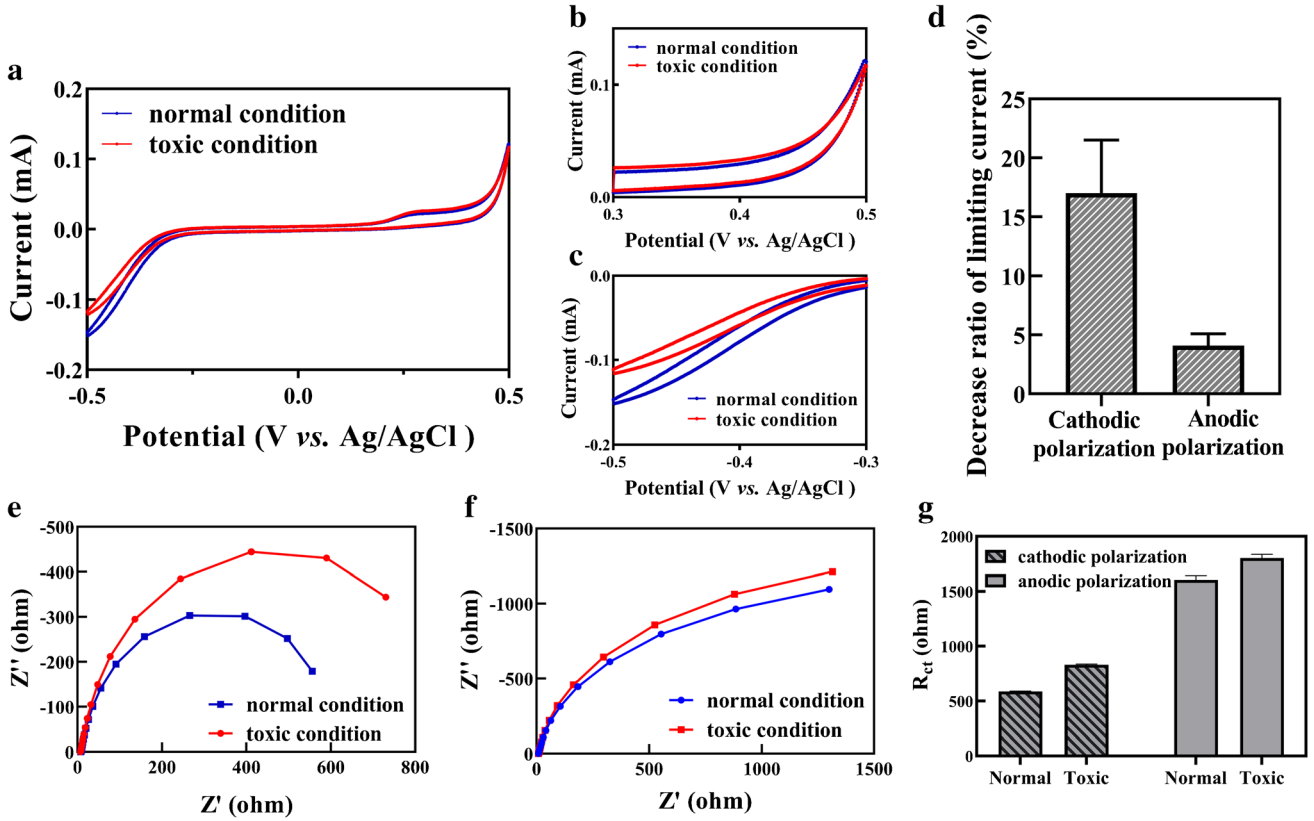

Fig. 5 CV curves and Nyquist plots. **a–c** CV curves of a MES before and after 30 min of 0.05 mg/L Cd^{2+} exposure. CV was performed with the potential range of − 0.5 to 0.5 V, and the scan rate of 1 mV/s. **d** Decrease ratios of the anodic and cathodic limiting currents after 30 min of 0.05 mg/L Cd^{2+} exposure. EIS of a MES before and after 30 min of 0.05 mg/L Cd^{2+} exposure was recorded under cathodic (**e**) and anodic (**f**) polarization conditions. EIS was performed with the frequency range of 50 mHz to 100 kHz, and small disturbance voltage of 5 mV. The set potentials were − 0.5 V and 0.5 V for cathodic (**e**) and anodic (**f**) polarization, respectively. **g** Changes of cathodic and anodic R_{ct} before and after 30 min of 0.05 mg/L Cd^{2+} exposure

Table 2 Results of orthogonal experiments

Number	Cell density (CFU/mL)	Electrode potential (V)	TMAO concentration (mM)	Inhibition (%)
1	1.8×10^8	−0.4	5	13.8
2	2.4×10^8	−0.4	10	18.3
3	3×10^8	−0.4	15	20
4	1.8×10^8	−0.45	10	19.9
5	2.4×10^8	−0.45	15	23.5
6	3×10^8	−0.45	5	14.4
7	1.8×10^8	−0.5	15	34.6
8	2.4×10^8	−0.5	5	22.5
9	3×10^8	−0.5	10	25.9
K_1	68.3	52.1	50.7	
K_2	64.3	57.8	64.1	
K_3	60.3	83	78.1	
k_1	22.8	17.4	16.9	
k_2	21.4	19.3	21.4	
k_3	20.1	27.7	26.0	
R	2.7	10.3	9.1	

Ranking: electrode potential > TMAO concentration > cell density.

K_i the sum of the evaluation indexes of all levels ($i = 1, 2, 3$) in each factor, k_i mean value of K_i, R the range between the maximum and minimum value of k_i.

sensitivity with inward EET remained unclear. In the study, pure cultured and suspended *S. oneidensis* MR-1 was utilized, and all the MESs used the same electrolyte. In this way, the direct effects of EET direction on the sensitivity were revealed with a MES capable of bidirectional EET. In addition, this study first confirmed that the enhanced sensitivity was because inward EET reflected metabolism activity completely. Nonetheless, the coexistence of organic matters and TMAO may deteriorate cathodic polarization. Therefore, it is suggested using an electrode as the sole electron donor in practical applications.

Orthogonal optimization to enhance the sensitivity with inward EET

Cell density, electrode potential, and electron acceptor concentration are three key parameters determining the input currents of EAB, which affect the sensitivity of water toxicity detection. The detailed effects of these three parameters on the IR of suspended *S. oneidensis* MR-1 were investigated by orthogonal optimization. As shown in Table 2, three parameters had significant effects on IR. Specifically, the *k* value of cell density increased from 20.1 to 22.8 with the decrease of cell density, indicating that the decrease of cell density was suitable for water toxicity detection, which was also consistent with the results in the recent study [12]. Higher IR was obtained with a lower-potential electrode and higher-concentration TMAO, which suggested that the efficient electron donor and acceptor benefited the detection of water toxicity. However, different results were reported in the previous study, which revealed that the decrease of organic matters enhanced the sensitivity of water toxicity detection when using EAB with outward EET as the sensing element [40]. Compared with the previous studies, the obvious difference indicated that the substrate concentration had completely different effects on the sensitivity with different EET directions. Notably, the baseline current of EAB was positively associated with the substrate concentration; only the sensitivity and baseline current of EAB with inward EET was capable of being enhanced simultaneously. By using range analysis, the order of effects of the three parameters on IR was as follows: electrode potential > TMAO concentration > cell density. Additionally, the highest sensitivity was obtained with a cell density of 1.8×10^8 CFU/mL, an electrode potential of −0.5 V, and a TMAO concentration of 15 mM.

Conclusion

The study revealed the effects of EET direction on the sensitivity of water toxicity detection based on suspended *S. oneidensis* MR-1, and further investigated the underlying mechanism. Compared with outward EET, inward EET improved the detection of low-concentration pollutants, and the sensitivity was enhanced at most 2.7 times. This was because inward EET includes all the electron transfer of the respiratory chain, and is capable of more effectively reflecting bacterial viability. The three key parameters of inward EET were optimized to further improve water toxicity detection. The highest sensitivity was obtained with a potential of −0.5 V, a cell density of 1.8×10^8 CFU/mL, and an electron acceptor concentration of 15 mM, which provides a guide for practical applications.

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Declarations

Competing interests The authors declare no competing interests.

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