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Short communication

Concentration responses of toxicity sensor with *Shewanella oneidensis* MR-1 growing in bioelectrochemical systems

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ABSTRACT

Bioelectrochemical systems (BESs) provide an opportunity to detect biological toxicity of water samples. However, the concentration responses of toxins had not been investigated in detail. Using formaldehyde as a toxic substance, the current responses were analyzed over a concentration range from 0.01% to 0.10% in a single chambered BES with 0 mV (versus saturated calomel electrode) applied on the anode. The decay percentages of currents increased in proportion with the concentration of formaldehyde after 10000 s (~2.8 h), with the peak R^2 of 0.9361 observed at 35,000 s (~9.7 h). Fitting results of exponential decay equation showed that the magnification factor (a) closely related with baseline currents and the toxicity factor (b) was in direct proportion to formaldehyde concentration (from 0% to 0.08%) except over the high concentration of 0.10%. These results provide preliminary information about toxin concentration responses in BESs.

1. Introduction

With the fast development of modern industry, emerging contaminations such as pharmaceuticals and personal care products (PPCPs) bring us new challenges and environmental problems since these pollutants cannot be traditionally monitored (Gatermann et al., 1998). Different pollutants, either traditional or emerging, mix and inter-react in the natural environment, forming more complicated contaminations called 'combined pollutants' or 'co-contaminations' (Sun and Zhou, 2007; Zhou, 1995; Zhou et al., 2008). The evaluation of the joint toxicity of these complex contaminations, especially in water environment, has not been well investigated.

Mediator-less microbial fuel cells (MFCs) had been invented in 1999 by Kim et al. as a lactate biosensor. They found that the current was in proportion with lactate concentration up to 30 mM (Kim et al., 1999), and provides a possible approach for *in-situ* monitoring of bacterial responses through a simple recording of current or voltage when the aqueous econiche changes. Although MFCs extensively bloomed in areas of electrical energy recovery from wastewater (Rozendal et al., 2008), conversion of waste into chemicals (Logan and Rabaey, 2012) and bioremediation (Wang et al,. 2012), the possible application of MFC as biosensor is still a promising direction worth to be investigated. It had been demonstrated that biological oxygen demand (BOD) of wastewater linearly related with the total Coulombs produced (Kim et al., 2003a), which had been utilized in the measurement of BOD in real wastewater, as a low BOD sensor or as an organic carbon sensor (Kang et al., 2003; Kim et al., 2003b; Peixoto et al., 2011). Since all these responses of current on substrate concentration were attributed to the variation of microbial activity (Tront et al., 2008), MFCs can be extensively utilized as toxicity sensors. Kim et al. (2007) found that toxic substances including an organophosphorus compound, Pb, Hg and polychlorinated biphenyls (PCBs) inhibited the current by 28–61% when 1 mg/L of each pollutant was injected individually. However, the toxin concentration (or addition times) exhibited a partly positive but irregular response to the inhibition ratio of the current, making this promising *in-situ* toxicity sensor deficient in predicting toxicity quantitatively. 0.1% of formaldehyde had been utilized as a toxin in a silicon-based biosensor, but the electrochemical response to the concentration of formaldehyde had not been investigated (Davila et al., 2011).

So far as we know, the relationship between current and toxin concentration still needs to be investigated. Since the soluble formaldehyde has a fast biological toxicity and stable with chemicals added in MFCs, it was selected here as the tested toxin. Concentration responses of formaldehyde were analyzed based on the time scale and current kinetics in single chambered poised potential bioelectrochemical systems (BESs) with *Shewanella oneidensis* MR-1 as exoelectrogenic bacteria.

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2. Materials and methods

2.1. Bacterial strain and growth conditions

Luria–Bertani (LB) broth prepared in 50 mM phosphate buffer solution (PBS, containing Na_2HPO_4 4.576 g/L, $NaH_2PO_4 \cdot H_2O$ 3.321 g/L, NH_4Cl 0.31 g/L and KCl 0.13 g/L) was used as growth medium and electrolyte during start-up period for *S. oneidensis* MR-1 as previous described (Peng et al., 2010a). Bacteria were inoculated in LB–PBS medium and incubated aerobically in a 30 °C shaking flask (150 rpm) for 48 h with the optical density (OD_{600}) reached ~2.4.

During the contaminating period, the electrolyte was switched to mineral medium containing: 50 mM PBS, 12.5 mL/L mineral solution (Lovley and Phillips, 1988) and 200 mM lactate to avoid possible interferents from the complex component of LB (Huang et al., 2011). The mineral medium was sparged with N_2 to remove oxygen before using. All the tests were performed in a 30 ± 1 °C temperature controlled room.

2.2. BES construction and operation

Single chambered poised potential BESs were utilized as toxicity biosensors. The BES was constructed using a 120 mL reagent glass with three electrodes inserted through the cap and sealed with silicon gel (Fig. S1A), including a graphite rod (diameter of 1 cm) as the working electrode, a Pt electrode (1 cm^2) as the counter electrode and a saturated calomel electrode (SCE, saturated KCl solution, 241 mV versus standard hydrogen electrode) as the reference electrode. Electrode spacing was fixed at 2 cm. Graphite rod was pretreated by 1 M HCl overnight and rinsed by DI water for three times. All these anodes were equipped in abiotic BESs and poised at +0.3 V (vs. SCE) in PBS for over 1 day to remove impurities before using.

When 60 mL of bacterial suspension was transferred into a sterile reactor containing 60 mL of LB–PBS medium, the potential of working electrode was poised at 0 mV (vs. SCE; 241 mV vs. SHE) using 8-channel potentiostat (CHI 1000B, Chenhua Instruments Co., Ltd., Shanghai, China) since *S. oneidensis* expressed cytochromes OmcA/MtrC at the bacteria–electrode interface at this potential (Peng et al., 2010b). The current of each BES was recorded every 5 s. Anodic bacteria were consecutively acclimated for 2 days to ensure a mature biofilm before the electrolyte was switched to sterile mineral medium. Reactors were substantially mixed using magnetic stirrer (Fig. S1B).

2.3. Formaldehyde injection

After ~1.3 h (4650 s) of acclimation in mineral medium, concentrated sterile formaldehyde (10%, PBS diluted) was injected into each sensor individually with final volume concentrations of 0.01%, 0.03%, 0.04%, 0.05%, 0.07%, 0.08% and 0.10%, with one reactor operated without any formaldehyde addition as the control. Currents were continuously recorded for 48 h after formaldehyde injection. After these measurements were finished, all these tests were repeated for one time in sterilized reactors. Decay percentage (p) was calculated as $p=(I_{bi}-I_i)/I_{bi}$, where I_{bi} (A) is the baseline current of BES before formaldehyde injection, I_i (A) is the current at time t and t (s) is the contact time after formaldehyde injection. Despite the physical significance, the current was also fitted by an exponential decay equation (Eq. (1)), where a is the magnification factor, b is the toxicity factor, 3600 s/h is the scaling factor.

$$I = a \times e^{-bt/3600} \quad (1)$$

3. Results and discussion

3.1. Baselines of single chambered BES sensors

Since anodic bacteria gain energy from overpotential, poising the anode potential makes the anodic electrochemical environment identical in each run. Current was immediately generated as soon as *S. oneidensis* MR-1 inoculated in each BES, with currents of $3.0 \pm 0.9 \times 10^{-5}$ A observed at 14 min. As indicated in Fig. S3, the negative current means electron output from working electrode according to the definition of the manufacturer. These currents were attributed to the oxidation of redox enzymes and soluble mediators in planktonic cells and the inoculated medium. As reported previously, stable current of $1.0 \pm 0.4 \times 10^{-4}$ A was obtained at 6 h in each reactor, indicating that MR-1 had colonized the surface of anode (Baron et al., 2009). The enrichment period was extended to 2 days to obtain mature biofilm. Currents slightly fluctuated and started to decline at the end of this period.

When substrate was switched into mineral medium, flat baselines were observed with current of $1.0 \pm 0.3 \times 10^{-4}$ A (Fig. 1A). It was interesting that the stable current produced from lactate and mineral medium was similar with those produced from LB–PBS, indicating that the current output in present study was not limited by the substrate or electrolyte. As addressed previously, a stable baseline needed a stable pH, saturated substrate and fixed anode potential (overpotential) (Stein et al., 2010). 200 mM of lactate was saturated since *S. oneidensis* MR-1 electrogenesis is proportional to fuel concentration but can be saturated at 100 mM lactate (Cho and Ellington, 2007). The pH variation of mineral medium in this test was <0.2, showing that the addition of phosphate buffer, the close-set (2 cm) of counter electrode, the continuous stirring and the membrane-less design of BES effectively eliminated the pH gradient and kept the baseline stable.

3.2. Toxic responses of formaldehyde concentration

As soon as different concentrations of formaldehyde were injected, currents of all these BESs exhibited attenuations except the control, showing that formaldehyde over the concentration

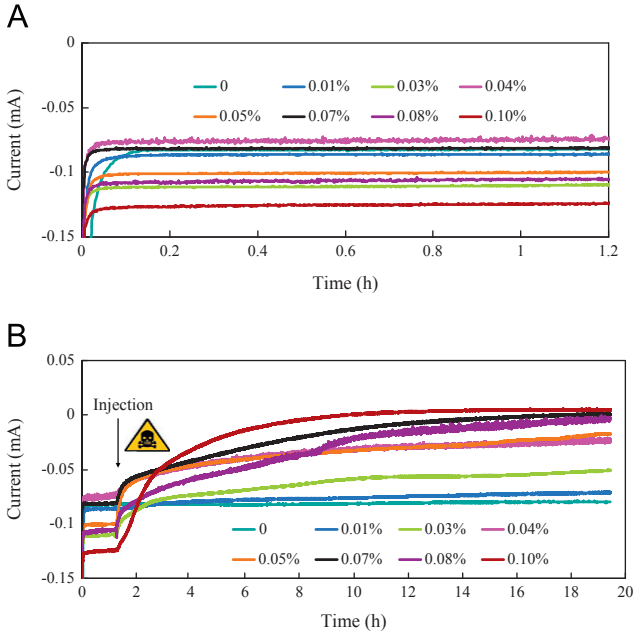

Fig. 1. Baseline currents (A) and current responses of formaldehyde concentrations (from 0.01% to 0.10%) with a BES as the blank control (B).