Supporting information for

An unexpected discovery of 1,4-benzoquinone as a lipophilic mediator for toxicity detection in water

Dengbin Yu,[a,b,d] Jiantao Li,[b] Zhichao Kang,[a,d] *Ling Liu,[b] Jingting He,[b]* Hongwen Yu*[a,d] and Shaojun Dong*[b,c]

[a] *Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Science, Changchun 130102, Jilin, P. R. China.*

[b] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Science, Changchun 130022, Jilin, P. R. China.*

[c] *University of Science and Technology of China, Hefei 230026, Anhui, P. R. China.*

[d] *University of Chinese Academy of Sciences, Beijing 100049, P. R. China*

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*Corresponding authors:

E-mail addresses: yuhw@iga.ac.cn; dongsj@ciac.ac.cn

Feasibility of toxicity detection with the biosensor

To demonstrate the feasibility of toxicity detection with the biosensor, the anodic changes of the composite-mediated respiration (CM-RES) of BQ–$K_3[Fe(CN)_6]$ activity of bacteria were measured as shown in Fig. S1. It should be noted that the final concentrations of cells, $K_3[Fe(CN)_6]$, BQ and Cu^{2+} were OD_{600} = 3, 18 mM, 10 μM and 1 mg/L, respectively. The solution sample d and sample e were incubated for 1 h at 37 °C on a shaker at 220 rpm.

It is clear from Fig. S1 that the detection time of each sample was less than 10 s, which is helpful for rapid detection. Anodic currents were not observed for BQ–$K_3[Fe(CN)_6]$ (Fig. S1A, curve a) or the mixture containing BQ–$K_3[Fe(CN)_6]$ and Cu^{2+} (Fig. S1A, curve b). The colors of the sample a and sample b in Fig. S1B are identical to that of $K_3[Fe(CN)_6]$, which indicates that Cu^{2+} cannot be oxidized by BQ–$K_3[Fe(CN)_6]$ or $K_3[Fe(CN)_6]$ at 450 mV. BQ–$K_3[Fe(CN)_6]$ can easily accept electrons from cells, as demonstrated by the immediate appearance of an anodic current after mixing the combined mediator with cells, as shown in Fig. S1A, curve c. Then, the resultant mixture was incubated for 1 h at 37 °C, and the anodic current increased dramatically because of the accumulation of $K_4[Fe(CN)_6]$ (Fig. S1A, curve d). The mixture containing BQ–$K_3[Fe(CN)_6]$ and cells was also incubated in the presence of Cu^{2+}; however, although the anodic current still increased, the current was smaller than that obtained in the absence of Cu^{2+} (Fig. S1A, curve e).

The color changes of each sample in Fig. S1B show agreement with the current changes in Fig. S1A, which further confirms the above conclusions. These results demonstrate that the CM-RES activity of cells can be inhibited by Cu^{2+}, and show the feasibility of the proposed bioassay in toxicity detection. In this way, a steady current was provided by the microelectrode array (MEA) in less than 10 s. The toxicity could be effectively quantified by the electrochemical method in Fig. S1A as well as qualitatively detected by the colorimetric process in Fig. S1B. From the former method, the inhibition ratio of 1 mg/L Cu^{2+} was calculated to be 6.96%.

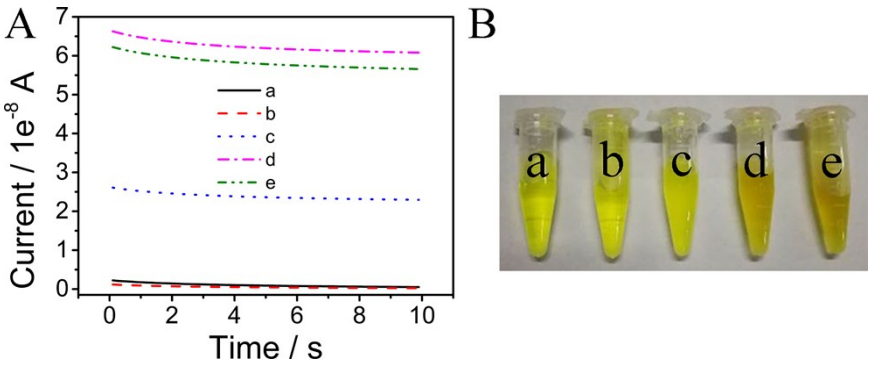

Fig. S1 (A) *i-t* curves of the mixed microorganismal suspension (OD_{600} = 3, BQ concentration = 10 μM, $K_3[Fe(CN)_6]$ concentration = 18 mM) used to assay the samples of (a) BQ–$K_3[Fe(CN)_6]$; (b) BQ–$K_3[Fe(CN)_6]$ and Cu^{2+} (1 mg/L); (c) BQ–$K_3[Fe(CN)_6]$ and mixed microorganisms (no cultivation); (d) BQ–$K_3[Fe(CN)_6]$ and mixed microorganisms (cultured for 1 h); and (e) BQ–$K_3[Fe(CN)_6]$, mixed microorganisms and Cu^{2+} (1 mg/L) (cultured for 1 h); (B) photo of samples corresponding to the *i-t* curves in Fig. S1A.

Comparison of the IC_{50} values

Table S1 Comparison of the IC_{50} values of Cu^{2+}, Cd^{2+} and Zn^{2+} obtained by the proposed method with those of mediated electrochemical microbial biosensors reported in literatures.

Toxicity assays	Microorganisms	Mediator	Heavy metal ions, IC_{50} (mg/L)			Ref.
			Cu^{2+}	Cd^{2+}	Zn^{2+}	
M-RES	*Mixed microorganisms*	$K_3[Fe(CN)_6]$	2.34	5.88	2.42	This
	Mixed microorganisms	BQ-$K_3[Fe(CN)_6]$	5.95	7.12	8.86	study
FM-RES	*Activated Sludge*	$K_3[Fe(CN)_6]$	-	13.42	1.189	[1]
Isolation method	*E. coli*	$K_3[Fe(CN)_6]$	21.3	3.7	26.7	[2]
FM-RES	*E. coli*	$K_3[Fe(CN)_6]$	3.71	7.8	7.8	[3]
ToxTell biosensor	*Psychrobacter sp*	BQ	2.6	47.3	10.9	[4]
BGSH biosensor	*E. coli*	BQ	44	79	-	[5]
Co-immobilizing	*E. coli, S. cerevisiae* and *B. subtilis*	BQ	16.5	20.5	-	[6]

-: No data
FM-RES: ferricyanide-mediated respiration
M-RES: mediated respiration
BGSH: benzoquinone/gelatin/silica hydrogel

References

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