

Accepted Manuscript

Dual-signal-biosensor based on luminescent bacteria biofilm for real-time online alert of Cu(II) shock

Xiang Qi, Pan-Pan Liu, Peng Liang, Wen Hao, Meng Li, Xia Huang



PII: S0956-5663(19)30579-2

DOI: <https://doi.org/10.1016/j.bios.2019.111500>

Article Number: 111500

Reference: BIOS 111500

To appear in: *Biosensors and Bioelectronics*

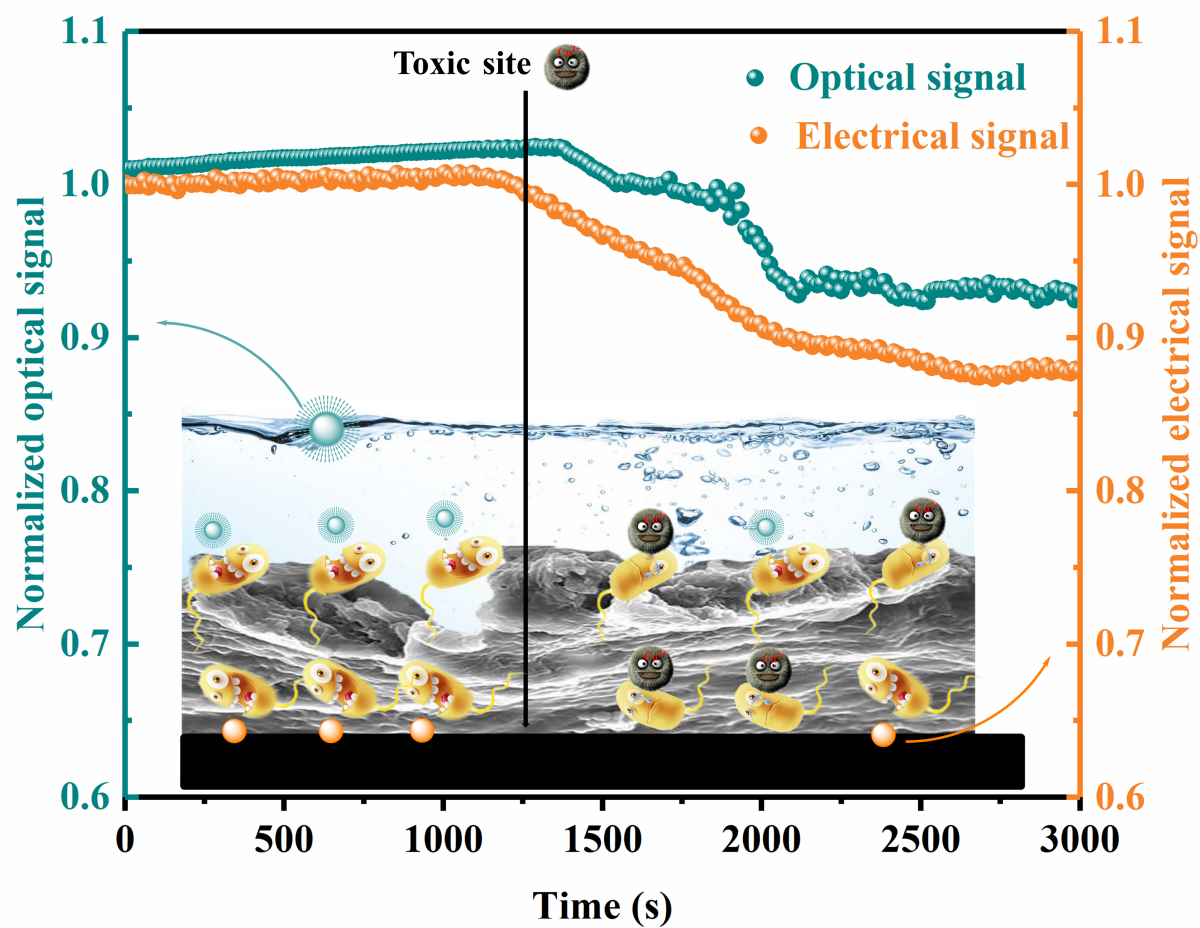
Received Date: 9 April 2019

Revised Date: 6 July 2019

Accepted Date: 12 July 2019

Please cite this article as: Qi, X., Liu, P.-P., Liang, P., Hao, W., Li, M., Huang, X., Dual-signal-biosensor based on luminescent bacteria biofilm for real-time online alert of Cu(II) shock, *Biosensors and Bioelectronics* (2019), doi: <https://doi.org/10.1016/j.bios.2019.111500>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Dual-signal-biosensor based on luminescent bacteria biofilm for real-time online alert of Cu(II) shock

Xiang Qi, Pan-Pan Liu, Peng Liang*, Wen Hao, Meng Li, Xia Huang

State Key Joint Laboratory of Environment Simulation and Pollution Control, School
of Environment, Tsinghua University, Beijing 100084, PR China

Abstract

The development of real-time online warning system for toxicity materials is important to ensure the safety of water supply. This study for the first time constructs luminescent bacteria (*Vibrio fischeri*) biofilm to deliver both electrical and optical real-time response for Cu(II) toxic shock in a bioelectrochemical system (BES) sensor. Compared to biocathode, bioanode was more suitable as sensitive elements. With three tested concentrations of Cu(II), i.e., 1 mg/L, 3 mg/L and 6 mg/L, electrical signals were raised. But optical signal failed to respond to the lowest concentration, suggesting that electrical signal then was produced by chemical reaction of Cu(II) on the electrode surface. For 3 mg/L and 6 mg/L Cu(II) shock, more rapid optical signals were observed than electrical signal, indicating that both the biofilm's surface and inner was affected. In addition, high concentration of Cu(II) toxic as 6 mg/L caused irreversible damage in the biosensor as there were great fluctuation in the recovery curve and large recovery ratio up to -10.39% for optical signal. These results provided a comparison between optical and electrical signals simultaneously produced by a biosensor and visual evidences for better understanding of the toxicity process in the biosensor.

Keywords: Biosensor, Biofilm, Optical and electrical signals, Cu(II), *Vibrio fischeri*

25

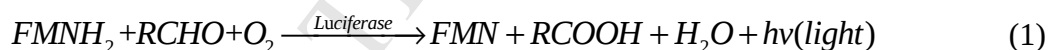
26 **1. Introduction**

27 Toxic pollutants (e.g. heavy metals) could enter into hydrosphere with the global
28 industrialization easily, and result in water contamination. Therefore, the frequent
29 monitoring of toxic pollutants is important to ensure the safety of water supply.
30 Toxicity monitoring of water quality mainly employ fish, daphnia, algae or
31 microorganisms for assays (Adekunle et al. 2019). Nevertheless, the existing
32 environmental monitoring technology usually spend long-time to analyze in the
33 laboratory, and it will fails to early alert of contaminants. Aiming to early warning the
34 toxicity, real-time on-line and on-site toxicity sensors have been studied in recent
35 years (Jiang et al. 2018; Lei et al. 2006), which is normally consisted of three
36 components: the sensitive element, the transducer and the detector. Different sensitive
37 elements have been used in toxicity sensors, including antibodies, organelles, cellular
38 receptors, enzymes, or even the whole cells (Parkhey and Mohan 2019). The redox
39 state will change while the sensitive elements suffer from the single (specific sensor)
40 or complex (broad-spectrum sensor) shocks of toxic materials (Shah et al. 2014).
41 After that, the produced signals are transformed into a transducer, the core component
42 of a biosensor. The transducer mainly depends on the character of bioelements and
43 comprise a variety of signals, including electrochemical signals, optical signals,
44 piezo/pyro electric signals and respirometric signals (Parkhey and Mohan 2019).
45 However, it is generally noticed that on-line toxicity sensors with the bioelements
46 mentioned above are normally inapplicable (e.g. expensive, difficult to operate and
47 maintain) during long-time operation with a continuous influent.

48 Bioelectrochemical system (BES) could produce electricity through electricigens
49 biofilm, and has been tested as a biosensor for water quality early-warning (Kim et al.

2007). Currently, BES sensor (e.g. microbial fuel cell sensor) is mainly considered as a broad-spectrum biosensor for sewage alert (Jiang et al. 2018). The performance of the BES sensor has been greatly improved through intensive studies on its sensing element, electrode material, operation mode and configuration (Jiang et al. 2017; Jiang et al. 2018; Liu et al. 2014; Xu et al. 2016; Zhao et al. 2019). Comparing to other technology, BES sensor have several advantages, such as inexpensive, easy to operate, maintain. But it is found that part of electrical signal produced by BES sensor is caused by the chemical reaction on the electrode surface rather than biotoxicity, which would result in inaccurate warning (Jiang et al. 2015). In addition, there is no international standard established on BES sensor and its sensing process.

As a comparison towards BES sensor, Luminescent bacteria (e.g. *Vibrio fischeri*) as the whole microbial cells are capable of emitting optical signal (blue-green light) through the oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain aldehyde ($RCHO$) by diatomic oxygen with Luciferase as the mediator (Inouye 1994), as expressed by



In fact, luminescent bacteria have been used to detect the biotoxicity of ecological environment for decades (Girotti et al. 2008; Ma et al. 2014). With a series of official standards issued, e.g. GB/T 15441-1995 in China, ASTM D5660-1995 in the U.S., DIN 38412-1990 in France, and EN ISO 11348 in European countries, this method is established worldwide to monitor the pollutants including inorganic pollutants, organic pollutants, general toxicity and the genotoxicity (Abbas et al. 2018). Luminescent bacterial suspension is a widely-used indicator for ecotoxicity detection, as it is cost-effective and is cultured rapidly. However, it fails to deliver real-time optical signal for online toxic alert due to the instability of luminescent bacterial

suspension (Ma et al. 2014).

This study for the first time employed luminescent bacteria (*Vibrio fischeri*) biofilm as the responsive element in BES sensor for real-time online alert of Cu(II) toxic shock. When the biofilm was applied as the bioanode, the biosensor raised alerts at three tested Cu(II) concentrations from 1 to 6 mg/L. A comparison was provided between the electrical signal that is produced by BES sensor with optical signal from Luminescent bacteria that has been employed by many international standards related to sensing process. How different concentrations of Cu(II) had biocidal effects of *Vibrio fischeri* biofilms was investigated and the biosensor's recovery performance after the toxic shock was examined.

2. Materials and methods

2.1 Materials

2.2.1 Bacterial culture

The freeze-dried luminescent bacteria (*Vibrio fischeri* NRRLB-11177) was obtained from China General Microbiological Culture Collection Center (CGMCC) (Beijing, China). The strain was cultured in a triangular flask (Thermostat incubator, 26 °C) at a temperature of 121 °C for 20 min using the sterile growth medium, which had a pH of 7 ± 0.2 , and contained 3% NaCl, 6.1 g/L $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 2.75 g/L $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 0.204 g/L $\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$, 0.5 g/L $(\text{NH}_4)_2\text{HPO}_4$, 3 mL glycerol, 0.5 g/L yeast extract, and 3 g/L peptone (Wei et al. 2018; Yu et al. 2018). After 18 hours, the cells in exponential phase were inoculated into a reactor described as follows for water quality alert experiment. The phosphate buffer (PBS) was removed in the process of water quality alert experiment, and the toxic pollutant was CuCl_2 in this study.

2.2.2 Biosensor set-up and start up

The biosensor device employed in this study comprised three parts: BES, electrical signal monitoring system and optical signal monitoring system (Figure 1a). A 15 mL-sized three-electrode system served as the BES reactor, in which a working electrode made of cylinder-shaped carbon felt (diameter: 1.5 cm in diameter and 0.6 cm in thickness; Sanye Carbon Co. Ltd., China) was placed at one third of the reactor for microorganism attachment (Figure S1). Near the working electrode, a saturated calomel electrode (SCE, +242 mV vs. standard hydrogen electrode, SHE; Leici Co. Ltd., China) was set as the reference electrode. Platinum (Pt) (Gaoss Union Co. Ltd., China) sheet was employed as a counter electrode. A square transparent glass (5 cm in length) was used as the top of BES reactor. The outlet was located below the transparent glass to avoid the attachment effect of microbe. An electrochemical workstation (Autolab, Metrohm Co. Ltd., China branch) was employed to monitor electrical signals, while a photomultiplier (PMT, HAMAMATSU Co. Ltd., Beijing, China) and a modular connector (HAMAMATSU Co. Ltd., Beijing, China) were employed to collect optical signal. The whole biosensor device was operated in a black box against external photons in the whole experiments.

To start up the biosensor device, 2 mL cells solution were inoculated into the reactor and recirculated at a constant flow rate of 5 mL/min and a hydraulic retention time (HRT) of 3 min, to facilitated rapid growth of the biofilm (Figure 1b). When the biofilm was formed, continuous flow operation was applied afterwards. Air was pumped into the influent to provide dissolved oxygen (DO) to fully support the growth of the bioluminescence. In addition, all experiments were carried out at room temperature.

2.1 Methods

Photon numbers and the current were considered as the parameters of optical

signal and electrical signal, respectively. These two signals were recorded every 15 seconds, and the total toxic time was 30 minutes. The normalized optical signal (NOS) is determined as

$$NOS = \frac{P_n}{P}$$

(2)

where P_n and P are recorded photon number and the average photon number in stable stage after feeding normal medium without toxic pollutions, respectively.

While the normalized electrical signal (NES) is determined as

$$NES = \frac{i_n}{i}$$

(3)

where i_n and i are recorded current and the current in stable stage after feeding normal medium without toxic pollutions, respectively.

Inhibition ratio (IR) is employed to evaluate the alert effects on both optical and electrical signal (IR_{NOS} and IR_{NES} , respectively), as determined by equation (4) and (5) (Jiang et al. 2017; Zhao et al. 2019).

$$IR_{NOS}(\%) = \left(\frac{NOS_{nor} - NOS_{tox}}{NOS_{nor}} \right) \times 100\% \quad (4)$$

$$IR_{NES}(\%) = \left(\frac{NES_{nor} - NES_{tox}}{NES_{nor}} \right) \times 100\% \quad (5)$$

where NOS_{nor} and NES_{nor} were the photon number and the current after feeding normal medium without toxic pollutions, respectively; NOS_{tox} and NES_{tox} were the photon number and the current after feeding normal medium with toxic pollutions, respectively.

In addition, recovery ratio (RR) for both optical and electrical signals are

calculated according to equation (6) and (7).

$$RR_{NOS}(\%) = \left(\frac{NOS_{nor_0} - NOS_{nor_k}}{NOS_{nor_0}} \right) \times 100\% \quad (6)$$

$$RR_{NES}(\%) = \left(\frac{NES_{nor_0} - NES_{nor_k}}{NES_{nor_0}} \right) \times 100\% \quad (7)$$

Where NOS_{nor_0} and NES_{nor_0} were the initial photon number and the current after feeding normal medium without toxic pollutions, respectively; NOS_{nor_k} and NES_{nor_k} were the k^{th} photon number and current after recovery.

The sensitivity of dual-signal-biosensor includes electrical signal sensitivity (ESS, $L\ mg^{-1}\ cm^{-2}$) and optical signal sensitivity (OSS, $L\ mg^{-1}\ cm^{-2}$), and the calculated method refer microbial fuel cell sensor (Di Lorenzo et al. 2014). The equations are as following:

$$ESS = \frac{\Delta NES}{\Delta C} \frac{1}{A} \quad (8)$$

$$OSS = \frac{\Delta NOS}{\Delta C} \frac{1}{A} \quad (9)$$

Where ΔNES and ΔNOS are the unit change in the NES and NOS after toxicity for 30 min; ΔC (mg/L) is the change in the concentration of Cu(II); A (cm^2) is the total area of anode materials.

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were performed with an electrochemical workstation (Autolab, Metrohm Co. Ltd., China branch).

3. Results and discussion

3.1 The biofilm formation and electrochemical activity in BES

Two milliliters of cells solution were inoculated into BES for biofilm formation (Figure S1). Some cells were observed to attach on the carbon felt the next day, and a

mature *Vibrio fischeri* biofilm was obtained after 5 days of inoculation as evidenced by an obvious difference in CV curves (Figure 1c) where the oxidation and reduction peaks for *V. fischeri* remained at -0.08 V and -0.38 V, respectively. In addition, the diffusion was weak as suggested by EIS plot (Figure S2), indicating again that the biofilm had formed. These results were in line with those reported previously (Tian et al. 2017).

3.2 The optimum electrode for biosensor device

The reduction and oxidation peaks for *Vibrio fischeri* biofilm indicated that the bacteria could serve as both the electron acceptor and donor. As the reduction peak was more obvious, the biofilm was tested first as the biocathode (electron acceptor). With an applied potential of -0.65 V, the optical signal started to increase with the time, while the electrical signal continuously weakened (Figure 2a). Optical signal is known to be produced by *Vibrio fischeri* with the presence of both organic matters and DO, while electricity's production requires merely DO. It was noticeable that once the organic matter was removed, the output of electrical signal recovered soon, but the optical signal declined (Figure S3). When DO was removed afterwards, both optical and electrical signal decreased. These findings suggested *Vibrio fischeri* biofilm as the biocathode failed to deliver synchronous optical and electrical signals.

On the contrary when *Vibrio fischeri* biofilm served as the bioanode (electron donor), both optical and electrical signals increased with the time (Figure 2b). This experiment was performed in duplicate with two freshly grown biofilms, while both biofilms delivered increasing optical and electrical signals (Figure S4). Specifically, optical signal (biophotons) was produced mostly from the surface of the biofilm, where considerable amount of DO was consumed (Figure 2c). With much reduced DO inside the biofilm, the biophoton's production was greatly restrained, but few O₂

were beneficial for the electrode to capture the bioelectronics and thereby improved the electrical signal. In conclusion, a mature *Vibrio fischeri* biofilm as the bioanode could deliver both optical and electrical signals in a biosensor (Figure S5).

3.3 Dual-signal-biosensor's responses to Cu(II) toxic shocks

When the NOS and NES curves remained stable (with a fluctuation less than 3%), Cu(II) toxic pollutants at different concentrations (1 mg/L, 3 mg/L and 6 mg/L) were tested, respectively. These experiments were repeated for twice with one biofilm and the results showed good reproducibility (Figure S6). The smallest concentration of Cu(II) (1 mg/L) caused a slow decrease in NES, but had no effect on NOS, indicating that 1 mg/L of Cu(II) have no biocidal effect at all on *Vibrio fischeri* biofilms (Figure 3a). This finding supported a previous study claiming the electrical signal output when the biosensor was shocked by toxic pollutant at considerable low concentration was caused by the chemical reaction of Cu(II) on the electrode surface, rather than the response of the biofilm (Jiang et al. 2015). The dual signals were restored afterwards to its original level, and then 3 mg/L Cu(II) toxic pollution was added into the system. The NOS responded to toxic pollutions immediately, followed by NES, suggesting that the biotoxicity took place in the biofilm now. When a high concentration of toxic pollutions as 6 mg/L Cu(II) shocked the biosensor, the NOS once again responded faster than NES. The fact that the biosensor delivered more sensitive optical signals as compared to electrical signals would be attributed to the understanding that optical signal came from the surface of the biofilm as mentioned previously (Figure 2c). Compared with the inner of the biofilm, the surface was more responsive to toxic substances. The biotoxicity mechanism is directly linked to the cellular metabolic status, which include the effects of electron transport chain. Therefore, the bioluminescence and extracellular electron transfer (EET) will be

influenced by the destruction of electron transport chain.

Regardless of different sensitivity and response time of optical and electrical signals for three tested concentrations of toxic pollutant, the dual-signal-biosensor raised alerts for Cu(II) concentration of above 3 mg/L. The average value of IR_{NOS} and IR_{NES} achieved 13.61% and 15.39%, respectively, for 3 mg/L Cu(II) toxic shocks at a response time of 30 min (Figure 3b). Although the planktonic *Vibrio fischeri* is more sensitive than biofilm in the process of toxicity alert, it is inapplicable in real-time online operation with continuous influent. In addition, the IR of both signals displayed a good linear relationship at different concentrations of Cu(II) toxic shocks ($R^2_{NOS}=0.99846$ and $R^2_{NES}=0.99888$) (Figure S7). The analysis of sensitivity based on the anode total area showed that optical signal ($0.81 \text{ L mg}^{-1} \text{ cm}^{-2}$) was more sensitive than electrical signal ($0.54 \text{ L mg}^{-1} \text{ cm}^{-2}$) in the range of 1 to 6 mg/L Cu(II) for biotoxicity. Although the dual-signal biosensor was considered as an integrated toxicity warning system for water quality, the monitoring of some polluted-water might require the selectivity which was still under exploration in BES sensor studies. Recently, some researchers used BES sensor to monitor volatile fatty acids during the anaerobic digestion process, and the core technology was anion exchange membrane (Jiang et al. 2019; Jin et al. 2016).

Regarding the recovery performance of the dual-signal-biosensor, both optical and electrical signals (NOS and NES) were able to well recover at 1 mg/L and 3 mg/L Cu(II) with a relative stable recovery curve (fluctuation less than 2%, Figure 3c) and a small recovery ratio of proximately zero (Figure 3d). However, when a high toxic concentration of 6 mg/L Cu(II) was applied, the recovery curve could not be restored to original status and the recovery ratio became much larger (-10.39% for RR_{NOS} and -2.91% for RR_{NES}). In addition, CV curves obtained after the toxicity revealed that 6

mg/L Cu(II) strongly inhibited *Vibrio fischeri* biofilm's bioelectrochemical activity (Figure S8). These findings suggested that 6 mg/L Cu(II) toxic shock led to an irreversible damage to the biosensor.

4. Conclusions

This study employed luminescent bacteria (*Vibrio fischeri*) biofilm as the responsive element in BES sensor for online alert of Cu(II) toxic shock. During startup period, *Vibrio fischeri* could form a biofilm on carbon felt that delivered electrochemical activity and bioluminescence simultaneously. This biofilm was then applied as the bioanode rather than the biocathode to fabricate the dual-signal-biosensor, and tested for its responses to Cu(II) toxic pollutants at different concentrations (1 mg/L, 3 mg/L, and 6 mg/L). In addition, the optical signal was more sensitive than electrical signal for biotoxicity. These findings suggested that the bioluminescence, employed by international standards as warning signal, could be regarded as a reference for the electrical alerts raised by the biosensor, especially for relative high concentration of toxic pollutants. In addition, the comparison of the two signals revealed how the biosensor responded to toxic pollutants.

Acknowledgement

This work was supported by the National Science Foundation of China (NSFC No. 51422810).

Appendix A. Supporting information

The Supporting Information is available free of charge on the XXX

The prototype of biosensor, EIS plot, Schematic illustration of bioluminescence and extracellular electron transfer (EET), and the characterization of biosensor for Cu(II) toxic shocks.

Corresponding Author

269 * Email: liangpeng@tsinghua.edu.cn

270 **ORCID**

271 PENG LIANG: 0000-0001-7345-0844

272 **Notes**

273 The authors declare no competing financial interest.

274 **References**

- 275 Abbas, M., Adil, M., Ehtisham-Ul-Haque, S., Munir, B., Yameen, M., Ghaffar, A.,
276 Shar, G.A., Tahir, M.A., Iqbal, M.J.S.o.t.T.E., 2018. Sci. Total Environ. 626,
277 1295-1309.
- 278 Adekunle, A., Raghavan, V., Tartakovsky, B.J.B., 2019. Biosen. Bioelectron. 132,
279 382-390.
- 280 Di Lorenzo, M., Thomson, A.R., Schneider, K., Cameron, P.J., Ieropoulos, I., 2014.
281 Biosen. Bioelectron. 62, 182-188.
- 282 Girotti, S., Ferri, E.N., Fumo, M.G., Maiolini, E., 2008. Anal. Chim. Acta 608(1),
283 2-29.
- 284 Inouye, S., 1994. FEBS Lett. 347(2), 163-168.
- 285 Jiang, Y., Chu, N., Zeng, R.J., 2019. J. Clean. Prod. 232, 1371-1378.
- 286 Jiang, Y., Liang, P., Liu, P., Wang, D., Miao, B., Huang, X., 2017. Biosen. Bioelectron.
287 94, 344-350.
- 288 Jiang, Y., Yang, X., Liang, P., Liu, P., Huang, X., 2018. Renew. Sust. Energ. Rev. 81,
289 292-305.
- 290 Jiang, Y., Liang, P., Zhang, C., Bian, Y., Yang, X., Huang, X., Girguis, P.R., 2015.
291 Bioresour. Technol. 190, 367-372.

- 292 Jin, X., Angelidaki, I., Zhang, Y., 2016. *Environ. Sci. Technol.* 50(8), 4422-4429.
- 293 Kim, M., Sik Hyun, M., Gadd, G.M., Joo Kim, H., 2007. *J. Environ. Monitor.* 9(12),
294 1323-1328.
- 295 Lei, Y., Chen, W., Mulchandani, A., 2006. *Anal. Chim. Acta* 568(1-2), 200-210.
- 296 Liu, B., Lei, Y., Li, B., 2014. *Biosen. Bioelectron.* 62, 308-314.
- 297 Ma, X.Y., Wang, X.C., Ngo, H.H., Guo, W., Wu, M.N., Wang, N., 2014. *Sci. Total*
298 *Environ.* 468-469, 1-11.
- 299 Parkhey, P., Mohan, S.V., 2019. *Microbial Electrochemical Technology*, pp. 977-997.
300 Elsevier.
- 301 Shah, P., Kaushik, A., Zhu, X., Zhang, C., Li, C.-Z., 2014. *Analyst* 139(9), 2088-2098.
- 302 Tian, X., Zhao, F., You, L., Wu, X., Zheng, Z., Wu, R., Jiang, Y., Sun, S., 2017. *Phys.*
303 *Chem. Chem. Phys.* Pccp 19(3), 1746.
- 304 Wei, S., Wang, F., Chen, Y., Lan, T., Zhang, S., 2018. *Environ. Sci. Pollut. R.* 25(26),
305 26504-26511.
- 306 Xu, Z., Liu, Y., Williams, I., Li, Y., Qian, F., Zhang, H., Cai, D., Wang, L., Li, B.,
307 2016. *Biosen. Bioelectron.* 85, 232-239.
- 308 Yu, Z., Zhang, J., Hou, M., 2018. *Chemosphere* 199, 122-129.
- 309 Zhao, T., Xie, B., Yi, Y., Liu, H., 2019. *Bioresour. Technol.* 276, 276-280.

310

311

312

313

Figures:

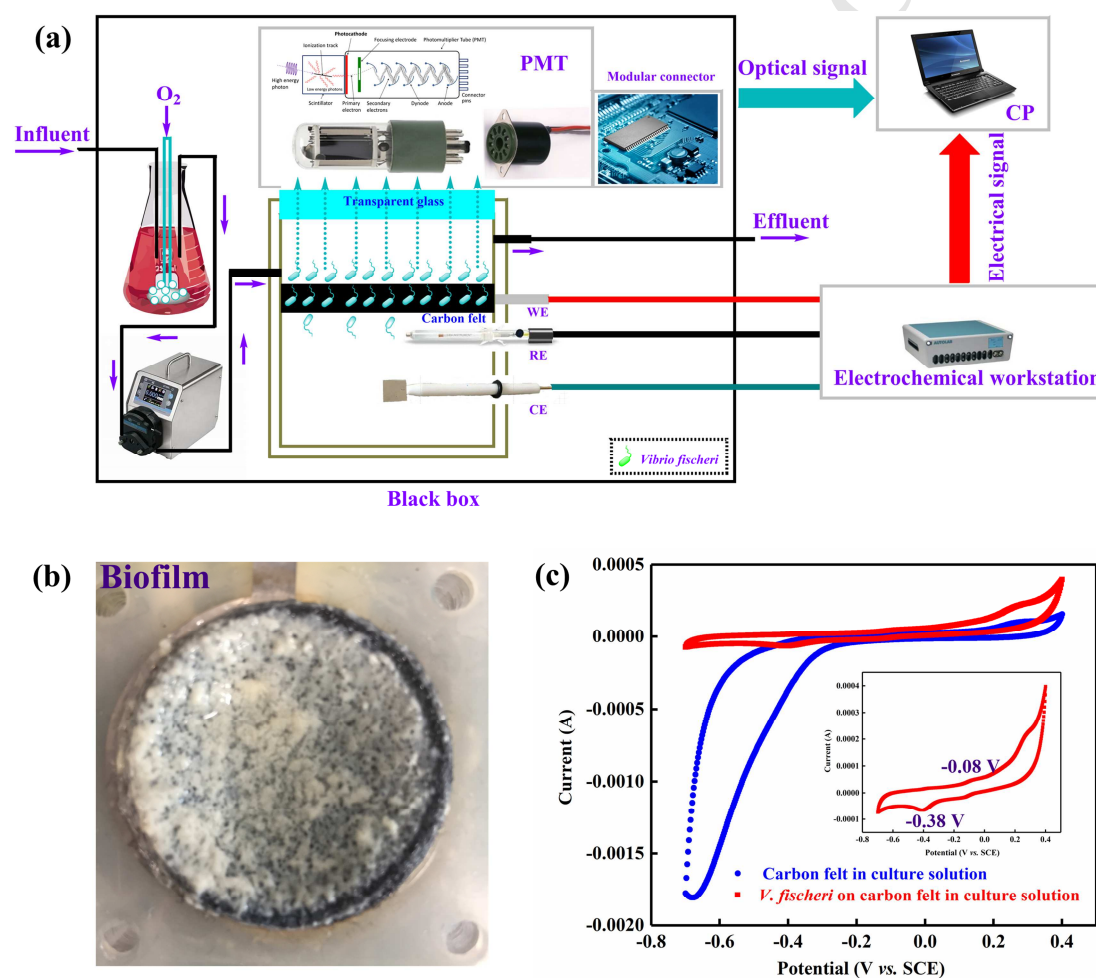
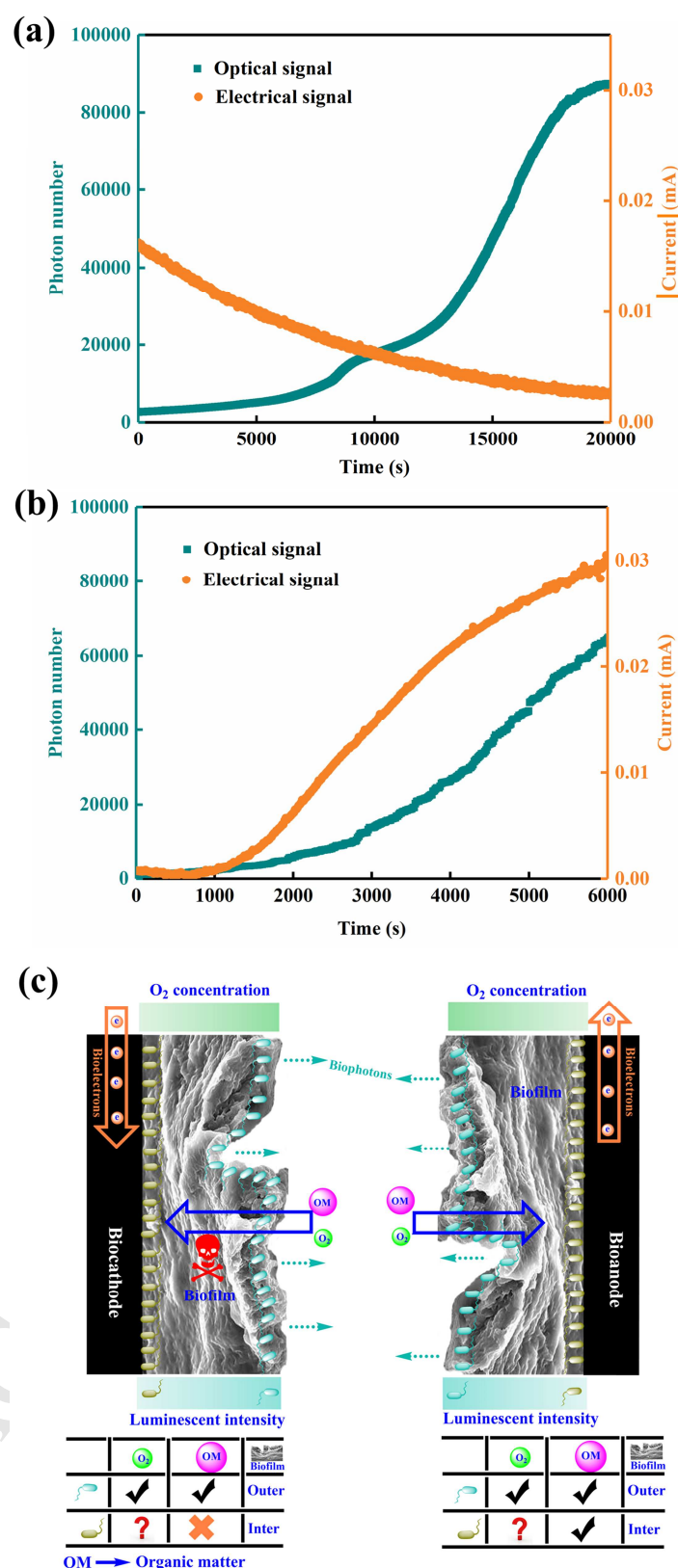


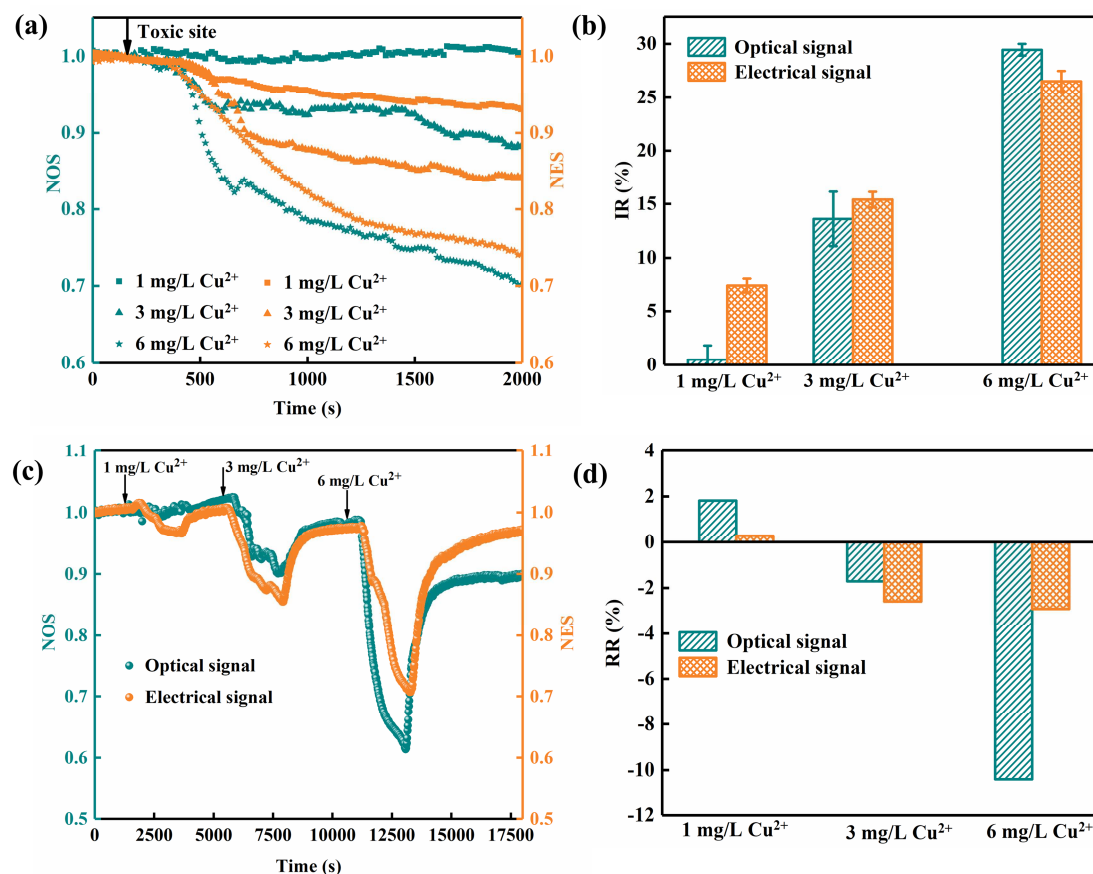
Figure 1. The biofilm formation and electrochemical activity in BES. (a) Schematic diagram of dual-signal-biosensor, (b) image of *Vibrio fischeri* biofilm, and (c) CV of *Vibrio fischeri* biofilm.



327

328 **Figure 2.** Performances of *Vibrio fischeri* biofilm as the biocathode and bioanode. (a)
 329 The change of optical and electrical signals with the time when *Vibrio fischeri* biofilm
 330 served as the biocathode (electron acceptor), (b) the change of optical and electrical
 331 signals with the time when *Vibrio fischeri* biofilm served as the bioanode (electron
 332 donor), and (c) the production of biophotons and bioelectrons by *Vibrio fischeri*

333 biofilm.



334

335 **Figure 3.** Dual-signal-biosensor's responses to Cu(II) toxic shocks within 30 min. (a)
 336 The responses to Cu(II) toxic shocks at different concentrations, (b) inhibition ratio, (c)
 337 recovery process, and (d) recovery ratio after toxicity.

Highlights

- Luminescent bacteria biofilm was constructed as a sensing element
- Simultaneously production of photons and electrons by *Vibrio fischeri* biofilm
- Compatible *Vibrio fischeri* biofilm as bioanode rather than biocathode with biosensor
- Both optical and electrical signals respond to Cu^{2+} shock

Credit Author Statement

The authors declared that they have contributed to this work. We promised that there is no any credit problem for the manuscript entitled, “Dual-signal-biosensor based on luminescent bacteria biofilm for real-time online alert of Cu(II) shock”.

Declaration of Interest Statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, “Dual-signal-biosensor based on luminescent bacteria biofilm for real-time online alert of Cu(II) shock”.