Biosensors and Bioelectronics 111 (2018) 97–101

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Open external circuit for microbial fuel cell sensor to monitor the nitrate in aquatic environment

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ARTICLE INFO

Keywords:
Microbial fuel cell (MFC)
Nitrate
Open circuit
Sensitivity
Mathematical model

ABSTRACT

This study employed an open external circuit, rather than a closed circuit applied in previous studies, to operate an microbial fuel cell (MFC) sensor for real-time nitrate monitoring, and achieved surprisingly greater sensitivity (4.42 ± 0.3–6.66 ± 0.4 mV/(mg/L)) when the nitrate was at a concentration of 10–40 mg/L, compared to that of the MFC sensor with a closed circuit (0.8 ± 0.05–1.6 ± 0.1 mV/(mg/L)). The MFC sensor operated in open circuit (O-MFC sensor) delivered much more stable performance than that operated in closed circuit (C-MFC sensor) when affected by organic matter (NaAc). The sensitivity of O-MFC sensor was twice that of C-MFC sensor at a low background concentration of organic matter. When organic matter reached a high concentration, the sensitivity of O-MFC sensor remained at an acceptable level, while that of C-MFC sensor dropped to almost zero. Challenged by a combined shock of organic matter and nitrate, O-MFC sensor delivered evident electrical signals for nitrate warning, while C-MFC failed. Another novel feature of this study lies in a new mathematical model to examine the bioanode process of nitrate monitoring. It revealed that lower capacitance of the bioanode in O-MFC was the major contributor to the improved sensitivity of the device.

1. Introduction

Microbial fuel cell (MFC) based biosensor employs exoelectrogen as the indicator organisms for organic matter and heavy metal ion detection , where the organic matter acts as electron donor (Logan, 2009) and heavy metal ion directly inhibits the activity of the exoelectrogen (Jiang et al., 2015). The nitrate can cause electrical signals in external circuit and be detected by an MFC sensor as well, as it can act as electron acceptor and compete for the electrons produced by organic matter (OM) oxidation on the anode (Sukkasem et al., 2008). Liu (Liu et al., 2014) constructed a single-chamber MFC sensor and observed a voltage drop in external circuit when 10 mg/L NO_3-N appeared in the anolyte. However, the voltage drop was too small (with a voltage drop ratio of~5%) to create a biosensor warning. Hiroyuki (Kashima and Regan, 2015) and Srinivasan (Srinivasan and Butler, 2017) also found that the appearance of the nitrate in anolyte could cause the current or voltage drop in external circuit of MFC sensor. But the response time was rather long (more than 24 h). In addition, they indicated that the concentration of OM greatly affected the decline in electrical signal: When the concentration of nitrate was fixed, the anolyte of MFC with high C/N ratio could hardly affect output voltage, while the low C/N anolyte caused a significant voltage drop to nearly zero.

Previous studies of MFC sensors all employed a closed external circuit for BOD, toxins and nitrate detection. An increased sensitivity of the sensors for BOD and toxin monitoring can be achieved by optimizing external resistance to get a large current output, which also leads to a high anode potential (100–200 mV) (Jiang et al., 2015). But for nitrate monitoring, the reaction rate would decrease with higher electrode potential applied (Pous et al., 2015), implying that the nitrate had limited ability to compete with the anode for the electrons, and would fail to deliver a clear electrical signal. In contrast, if MFC sensor is operated with an open external circuit, the nitrate can get electrons fast because the anode potential is low enough. The disconnection of external circuit also cuts off the way the electrons transfer from anode to cathode so that the electrons would gather on the anode, and be available for the nitrate. Given these considerations, the MFC sensor operated with open external circuit is expected to deliver high sensitivity for nitrate warning. What's more, MFC uses organic matters as the "fuel", which means the concentration of organic matter will greatly affect the external circuit voltage/current. But hardly could organic matters influence open circuit voltage (Pasternak et al., 2017), implying that open external circuit would help maintain a stable performance of MFC sensor when the organic matter's concentration changes.

Mathematical model provides another insight besides experimental research into MFC devices to better understand its performance, but has rarely been applied on MFC sensors. Stein (Stein et al., 2011)

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https://doi.org/10.1016/j.bios.2018.04.018
Received 4 October 2017; Received in revised form 26 March 2018; Accepted 8 April 2018
Available online 10 April 2018
0956-5663/

introduced a kinetic model to study toxicity detection by an MFC biosensor. This model focused on the signal differences between the cyclic voltammetry (CV) curves before and after introducing the toxicity, rather than the dynamic change of current/voltage with the time. Xin (Wang et al., 2013) applied an empirical exponential equation to fit the dynamic process of current change with the time during the toxic process. Those models were both established for MFC sensors operated with closed circuit.

In this study, an MFC sensor was operated with an open external circuit for real-time nitrate monitoring. As a comparison, another MFC sensor was operated with a closed external circuit. The performances of both MFC sensors were investigated, as well as their anti-interference ability against OM and other electron acceptors. Besides experimental studies, a mathematical model was established to understand the sensing process of the nitrate through MFC sensor with open circuit.

2. Materials and methods

2.1. Construction of the microbial fuel cell

Two typical two-chamber MFC with the same structure were constructed as the nitrate sensors. The anode and cathode chamber has a liquid volume of 7 mL and 28 mL, respectively, and were separated by a cation exchange membrane (CMI7000, Membranes International Inc., USA). The anode was made of cylinder-shaped graphite felt (Sanye Carbon Co. Ltd., Beijing, China) with a dimension of 3 cm in diameter and 0.6 cm in thickness. The cathode was a carbon-fiber brush of 3 cm in length and 3 cm in diameter. A saturated calomel electrode (SCE, 242 mV versus a standard hydrogen electrode; Leici Co. Ltd., Shanghai, China) was inserted near the anode as the reference electrode. All potentials presented below were normalized to the standard hydrogen electrode (SHE). A flow-by influent mode of the anode was applied as described in previous study (Jiang et al., 2015).

2.2. Startup of MFC biosensors

The two MFCs were both inoculated with the effluent of an acetate-fed MFC which was enriched from anaerobic digestion sludge at the beginning and operated for more than seven years in our laboratory (Jiang et al., 2016, Zhang et al., 2016). It was found that the *Geobactor* was the dominating functional electro-microorganism. After long-time operation, it had been proved that mainly functional electro-microorganism like *Geobactor* took more than 40% of the whole microbial consortiums (Sun et al., 2011). During the start-up, they shared the same anolyte and catholyte. The anolyte was prepared by dissolving 1.64 g sodium acetate (NaAc), 0.31 g NH_4Cl_4, 4.4 g KH_2PO_4, 3.4 g $K_2HPO_4{\cdot}3H_2O$, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.1 g $MgCl_2{\cdot}6H_2O$, 12.5 mL trace minerals solution, and 5 mL vitamin solution in 1 L deionized water (Lovley and Phillips, 1988). The catholyte contained16.64 g $K_3Fe(CN)_6$, 4.4 g KH_2PO_4, and 3.4 g $K_2HPO_4{\cdot}3H_2O$ in 1 L deionized water. Both the anolyte and catholyte were recirculated at a constant flow rate of 5 mL/min by using a multichannel peristaltic pump and replaced every three days. Nitrogen gas was purged to the anolyte before the replacement in order to exclude the interference of dissolved oxygen (DO). Both MFCs were operated with closed external circuit at a fixed resistance of 1000 Ω until the external voltage didn't drop after the replacement of the anolyte. After that, one MFC (C-MFC) was still connected with a closed circuit at a fixed resistance of 400 Ω, while the other (O-MFC) was connected to an open circuit by breaking the previous external circuit. These two MFCs were fed with the anolyte containing (per liter) 0.164 g NaAc (2 mM) while other constituents remained the same as previously described, and operated for at least one week.

2.3. Biosensor test

After the startup, both MFC sensors were tested for their electrical signals towards the nitrate and organic matter, as well as a combined pollutant. Considering the response time of MFC sensor operated with closed external circuit was rather long as reported previously (Kashima and Regan, 2015), the following experiments employed 30 min as the reaction time as previous studies did (Jiang et al., 2017a, 2017b).

The two MFCs were firstly tested with the anolyte containing 0.0607, 0.1213, 0.01821, and 0.2428 mg/L sodium nitrate (approximately 10, 20, 30, and 40 mg/L nitrate), respectively. Fresh medium without any nitrate was introduced to the MFC sensor for recovery before applying a different concentration of the nitrate. Then the concentration of NaAc contained in the anolyte was changed to 0.082 g/L (1 mM), and both MFC sensors were operated for 3 days, during which the anolyte was prepared and replaced on a daily basis. After that, the two MFC sensors were tested with the anolyte containing 10, 20, 30, and 40 mg/L nitrate, respectively. At the end of the test, the concentration of NaAc increased to 0.41 g/L (5 mM) in the anolyte and the two MFC sensors were tested with different nitrate concentrations mentioned above.

Finally, both MFC sensors were challenged with a combined pollutant of organic matter/nitrate by changing the concentration of the acetate and nitrate simultaneously. Sulphate (SO_4^{2-}) was applied as a competing electron acceptor to examine the stability of MFC sensors. The tested anolyte (2 mM NaAc) contained 0.1479, 0.2958, 0.4437, and 0.5916 mg/L sodium sulphate (approximately 100, 200, 300, and 400 mg/L nitrate), respectively. No fresh medium was applied for recovery here.

2.4. Calculations

During the experiments, the voltages (output voltage of C-MFC, open circuit voltage of O-MFC) and the anode potentials were recorded every 5 s using a data acquisition system (DAQ2213, ADLINK, Beijing, China). The baseline of MFC sensor is defined as the output voltage of MFC before the toxin shock. The sensitivity is usually defined as the voltage change (ΔV, mV) per unit change of nitrate concentration (Δc, mg/L), given by Eq. (1) (Chouler and Di Lorenzo, 2015). *IR* represents the voltage drop ratio and is calculated as the percentage of anode potential drop (ΔU_a) normalized to the absolute value of anode potential before the exposing of nitrate, given by Eq. (2).

$$sensitivity = \frac{\Delta V}{\Delta c} \tag{1}$$

$$IR = \frac{\Delta U_a}{|U_a|} \times 100\% \tag{2}$$

3. Results

3.1. Sensitivity of MFC sensor to the nitrate

Considering that 10 mg/L nitrate is the upper limit of drinking water standards, the tests began with this concentration of the nitrate (Lockhart et al., 2013). The voltage drop ratio of C-MFC (20.48 ± 1.025%) was much higher than O-MFC's (3.125 ± 0.139%, approximate to that reported in Liu's research (Liu et al., 2014)) in 30 min experiment (Fig. S1), revealing that O-MFC delivered clear electrical signals for the nitrate while C-MFC failed. Then the concentration of the nitrate was gradually increased to 40 mg/L, while the voltages of both MFC sensors dropped (Fig. 1). By applying Eq. (1), the sensitivity of O-MFC sensor (4.42 ± 0.29–6.66 ± 0.36 mV/(mg/L)) was greatly improved compared to C-MFC (0.8 ± 0.035–1.6 ± 0.088 mV/(mg/L)).

3.2. Effect of organic matter (NaAc) on MFC sensor's sensitivity

The sensitivity of the MFC sensors would be determined by the