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Development of a sediment microbial fuel cell-based biosensor for simultaneous online monitoring of dissolved oxygen concentrations along various depths in lake water

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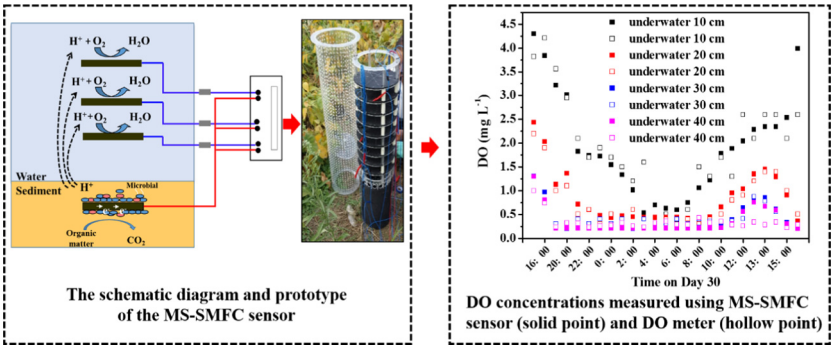
HIGHLIGHTS

- A novel sediment microbial fuel cell-based biosensor was developed.
- The sensor could online monitor the DO in different depths of lake water.
- The optimal anode to single cathode area ratio was 11:1.
- Organic matter contents in sediment affect the sensor operation performance.
- The sensor could be used as an early-alert program in a shallow lake.

GRAPHICAL ABSTRACT



The schematic diagram and prototype of the MS-SMFC sensor

DO concentrations measured using MS-SMFC sensor (solid point) and DO meter (hollow point)

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ABSTRACT

A novel multi-cathode, single-anode system integrating a sediment microbial fuel cell -based biosensor was developed for *in-situ*, continuous, and online monitoring of dissolved oxygen (DO) concentrations along various depths of lake water. The signal feedback mechanism was evaluated based on a relationship between voltage and DO concentration at corresponding depths. With an external resistance of 1000 Ω, a linear relationship was found (regression coefficient, $R^2 = 0.9576$) between voltage and DO in the range of 0–9 mg L^{-1}. The sensor performance was further optimized under various influence factors. The results of indoor experiments indicated that the optimal anode to single cathode area ratio was 11:1. The sensor signal could also be significantly influenced by organic matter content in sediment; thus, the addition of 5% organic matter could obtain a stable anode potential and a high voltage output. Furthermore, the sensor was operated *in-situ* for 67 days in a lake environment, which also led to a good correlation between the voltage and DO ($R^2 = 0.8897$). Thus, this integrated system has great potential as an early-warning program to help identify environmental risks in aquatic environments.

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1. Introduction

Dissolved oxygen (DO) refers to the level of free, non-compound oxygen present in water or other liquids. The vertical distribution of DO concentration is a fundamental property of aquatic ecosystems (Wilkinson et al., 2015). The phenomenon of DO concentration stratification in water column has been widely observed in marine and deep lakes (Wilkinson et al., 2015; Ortega-Retuerta et al., 2017). However, in shallow freshwater hypereutrophic lakes, like Taihu Lake in China, periodic DO stratification has also occurred in recent years (Zhu et al., 2008). One of the reasons is that, with a reduction of water transparency and effective light intensity, photosynthetic oxygen production capacity decreases as the depth of water increases (Zhang et al., 2012). Another important factor is that eutrophication induces an increase in the amount of organic matter in freshwater lakes, especially for the shallow lakes. Microbial decomposition of these organic materials results in the depletion of the DO concentration, and eventually leads to the risk of the lake generating "dead zone" (Shen et al., 2014). In fact, the dead zone is often generated first at the interface of water-sediments, as abundant organic matter are accumulated at the surface sediments (Zhang et al., 2010; Service, 2004). In general, degradation of excess organic matter led to the stratification in the eutrophication shallow lake. Thus, monitoring DO concentrations in the water columns of lakes is one of the key parameters for understanding the lake ecosystem. It could also be used as an early warning system for the "dead zone" risk in lake.

The traditional methods for DO determination are mainly physical, chemical, and electrochemical (Chen et al., 2007; Zhang and Angelidaki, 2012a). Some previously developed DO sensors, such as titration, amperometric, chemi-luminescent sensors, and others, are widely employed to measure the DO concentration in environmental areas (Kwak et al., 2014). However, these electrode type sensors have several drawbacks, such as expensive electrode materials (*e.g.*, gold), miniaturization difficulty, and electromagnetic interferences from other sensors (Kwak et al., 2014). Zhang and Angelidaki (2012b) designed the first MFC sensor for DO monitoring in aquatic environments using only one chamber structure. Although the sensor could be applied for *in situ* and real time monitoring DO, it might need to employ multiple-unit MFC systems for determining the DO concentration along the water depth in aquatic environments. Therefore, realizing simultaneous online monitoring in various depths of the water column in aquatic environments, as well as overcoming the drawbacks of traditional ones, are critical factors in the development of efficient and cost-effective DO sensors.

The microbial fuel cell (MFC) is a type of biological electrochemical device. MFC can convert chemical energy into electricity directly *via* bioprocesses which are catalyzed by exoelectrogenic microorganisms (Jia et al., 2016). Since voltage can be monitored easily online and consequently be converted into a signal which can be received by mobile phones, the MFC can be used as an inexpensive, dependable online biosensor (Jia et al., 2016; Jin et al., 2017). Compared to other kinds of biosensors, the main advantages of MFC are real-time monitoring and portability. Recently the MFC-based biosensor was successfully developed to determine biochemical oxygen demand (BOD) (Modin and Wilen, 2012), chemical oxygen demand (COD) (Jia et al., 2016), volatile fatty acids (Kaur et al., 2013), and toxicity (Wang et al., 2013) of water samples.

A sediment microbial fuel cell (SMFC) is a type of MFC. It has an anode electrode which is embedded in anaerobic sediments and a cathode electrode suspended in the aerobic water column above the sediment (Song et al., 2015). For most SMFCs, the cathode completes the circuit through the oxygen reduction reaction, in which DO is reduced to water (Hamelers et al., 2010) (Eq. (1)). In theory, the number of electrons transferred to an anode by microbes should correspond to electrons reacting with oxygen at the cathode. Thus, the voltage generated could also be a measure of DO concentration. The DO concentration can dramatically vary by depth in a lake. So, we speculate that if there were several cathodes along the lake depth, different voltages could be produced at various levels along the depth of lake water; then, the DO concentrations at different depths of lake water could be monitored.

$$O_2 + 4H^+ + 4\,e^- \rightarrow 2H_2O \left(E^0 = +805\ \text{m}\ V_{SHE}\ \text{for pH 7}\right) \quad (1)$$

Based on the above hypothesis, a multi-cathode, single-anode (MS) sediment microbial fuel cell (SMFC), hereafter referred to as an MS-SMFC, was employed as a DO sensor. The aim was to develop a more agile and simple system. The performance of the MS-SMFC sensor was optimized in terms of several indoor simulation experiments. The effect of key factors (*e.g.*, area ratio of anode and single cathode, organic matter content in sediments) on the sensor performance was explored. Practical application was also evaluated in a shallow lake. This strategy provided a sensitive detection platform for monitoring the DO concentration distribution pattern along the water depth in aquatic environments, and expanded the application of MFCs technology.

2. Materials and methods

2.1. Sediment, water and macrophyte samples

Surface sediments (at 0–10 cm depth) and water samples were collected from Taihu Lake (31°10′ N, 120°24′ E) (see Supporting Information, Fig. S1A). Taihu Lake is a large, shallow, eutrophic lake with a surface area of 2338 km^2 and a mean depth of 1.9 m (Song et al., 2016). Samples were transported to the laboratory as soon as possible. Then, the sedimentary samples were sieved through a standard 2-mm mesh screen and water samples were pre-filtered through a 200-μm-mesh to remove zooplankton and large detritus. Characteristics of the sediment sample were as follows: moisture, 48.1%; total nitrogen, 950.3 mg kg^{-1} dry sediment; total phosphate, 851.3 mg kg^{-1} dry sediment; total organic carbon, 16.4 g kg^{-1} dry sediment. Meanwhile, the properties of the water were measured as follows: pH 7.7, and conductivity 0.48 mS cm^{-1}.

As the major component of macrophytes was refractory lignocelluloses, the degradation rate of detritus was slow. In order to determine the effect of organic matter contents on the performance of the sensor, one of the most dominant submerged plants-*Potamogeton malaianus* (*P. malaianus*) in Taihu Lake, were also sampled. The plants were then crushed after air-drying and sifted through a 0.45 mm sieve.

2.2. Lab-scale single-cathode SMFC sensor operation

The lab-scale single-cathode SMFCs, which were made of nonconductive polycarbonate plates, were constructed as previously described in detail by Song and Jiang (2018). Briefly, a rectangle anode and a circular cathode were made of graphite felt (5 mm in thickness, Nengkang Carbon, Shanghai, China). Graphite felt is adopted as a typical electrode material because of its wide operating potentials, high corrosion resistance, good electrical conductivity, large porosity and low cost (Y. Jiang et al., 2017). The anode was placed approximately 5 cm below the surface of sediments and the cathode was put in the overlying water. A 1000 Ω external resistance was used to connect the anode and cathode *via* epoxy-encapsulated wires (Zhang and Angelidaki, 2012b).

After one month of continuous operations with a stable voltage and anodic performance, the overlying water was alternately poured into air or nitrogen gas, achieving a DO concentration in the range of 0 to 10 mg L^{-1}. Single-cathode SMFCs were first utilized to study the effect of the DO concentration on voltage generation using two tests: a long-term test (240 h) and a short-term test (12 h). Then, effects of single and multi-cathode structure on voltages output were compared (Fig. S2). The spacing between cathode and anode was set 20 cm both in the single and multi-cathode configuration. A DO probe was

put in the overlying water about underwater 5 cm and both oxygen concentration and voltage levels were monitored.

2.3. Lab-scale MS-SMFC sensor design

A lab-scale MS-SMFC was the reactor of the multi-cathode and single-anode structure (Fig. 1A and B). The numbers of cathodes and distances between the adjacent cathodes could be adjusted according to the actual needs. Each cathode which was connected to the same anode used a copper wire, and each external circuit contained a resistance of 1000 Ω. Furthermore, equivalent electrical circuit of the sensor was shown in Fig. S3. In the structure, the multiple cathodes were connected in parallel. All exposed metal surfaces were sealed with silver and nonconductive epoxy resin. In the experiments, the dimension of the anode was 40.8 cm × 30 cm (length × width) and the diameter of the cathode was 12 cm. In the indoor experiments, anode and cathode of MS-SMC were connected to a data acquisition/switch unit (model 2700, Keithley Instruments, Cleveland, OH, USA) and voltage data were collected every 30 s.

Before starting up the MS-SMFC, the anode was buried about 5 cm below the sediments, and the multiple-cathode was immersed in the water column, while the overlaying water was aerated with air pumps. To start up the system, the MS-SMFC was first operated for about 30 days to enrich electrochemically active biofilm and to get stable voltage and anode potential. All the MS-SMFC tests were run after the sensor showed higher and more stable voltage. During the test period, purified water was added as needed to maintain the water level.

First, a control experiment was done indoor to determine whether the sensor could respond to the DO changing along various depths of water (Fig. S4). About 40 cm sediment and 160 L lake water were put into a big barrel 105 cm × 60 cm (height × diameter). Plant litter was added in the sediments to maintain the organic matter content. The depth of the water column in the barrel was about 40 cm. The eight cathodes were designed in the indoor MS-SMFC system and the distance between adjacent cathodes was 5 cm. After one month of continuous operations with a stable voltage, N_2 was piped at the bottom of the water using aerator, and then different DO concentrations along the depths of water were obtained. The actual DO concentration at the different depths of water was also monitored using DO probe every 1 h. The DO probe was placed in the same position as the corresponding water depth of the cathodes.

Then, a series of experiments were conducted to optimize the MS-SMFC performance for high and stable anode potential and voltage generation. All experiments were carried out in triplicate. The MS-SMFC system contained 3 cathodes and the distances between adjacent cathodes were 5 cm. Experiment 1: The effects on voltage outputs of an anode to a single cathode area ratio were compared. Chosen ratios were 1.8:1, 3.6:1, 7.2:1, 11:1, and 14.5:1 by reducing the size of the cathode. Experiment 2: The effects of organic matter content in sediments on voltage outputs and anode potential were compared. Crushed plant litter was added into sediments, to adjust organic matter contents in sediments. Different contents of litter (*P. malaianus*), according to the litter/sediment ratios (dry weight/dry weight) were added to the sediments and mechanically mixed. The organic matter contents in sediments were 2%, 2.5%, 3%, 4%, and 5%, respectively.

2.4. In-situ experiments

The *in-situ* experiments were implemented in Eastern Taihu Bay (31°05′ N, 128°48′ E) of Suzhou (Fig. S1), which was fully covered by

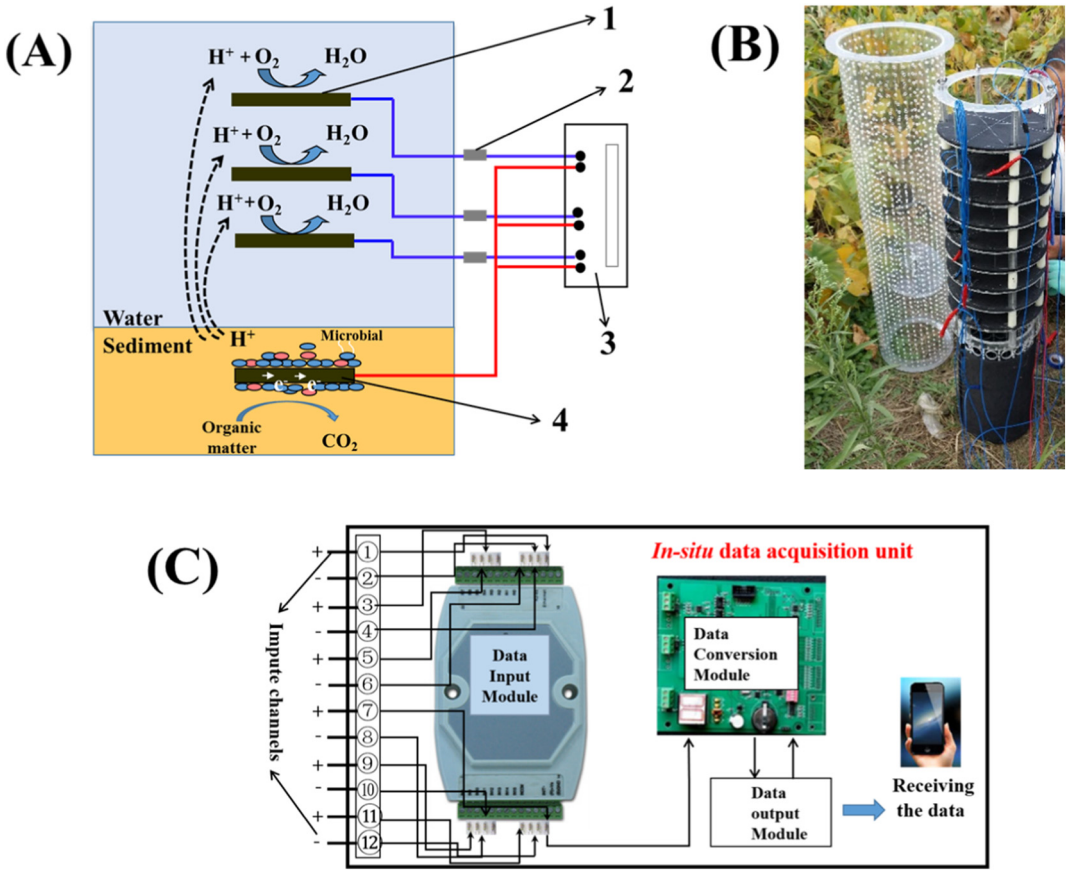

Fig. 1. Experimental set-up: (A) MS-SMFC sensor schematic diagram; (B) MS-SMFC sensor photograph; (C) *In situ* data acquisition unit; (1) cathode; (2) external resistance; (3) data acquisition system; (4) anode.

a submersed macrophyte community comprised mainly of *Hydrilla verticillata*, *Vallisneria spiralis* and *Potamogeton* spp. (Mao et al., 2014). Indeed, terrestrialization has been become serious in this bay mainly because of the accumulation of macrophyte detritus (Song et al., 2015). Furthermore, accumulated organic matters also caused the depletion of oxygen and accelerated the production of greenhouse gases such as methane (Song et al., 2015). Water depth at the site of the *in-situ* experiment was about 100 cm. The DO concentration was below 0.5 mg L^{-1} in the water of 30 cm down and showed an anaerobic condition. So, four cathodes were connected in the *in-situ* MS-SMFC system, and the distances between adjacent cathodes were 10 cm. Before starting the application, different depths of lake water and surface sediments were sampled and transported to the laboratory for analysis of the basic physicochemical properties. In the *in-situ* experiment, the anode and cathode were connected to the *in-situ* data acquisition unit, which contained input channels, a data input module, a data conversion module, and a data output module (Fig. 1C). The *in-situ* data acquisition unit was a homemade product and powered by extra power supply. It could set up the interval time of collecting data from 30 s to several hours, and the *in-situ* acquisition unit in this study logged the data every 30 min considering the storage ability of the unit. The *in situ* experiment was conducted in August and September in 2016. As the experiment site has a typical monsoon climate and the temperature difference between day and night is small in summer. Also, a previous study found that the vertical difference of water temperature in Taihu Lake was generally ranged between 0 and 1 °C (Zhao et al., 2011). So the surface water temperature was measured every two days.

2.5. Analysis and calculations

DO concentration was measured using a DO meter (Pro Plus, YSI, US). Prior to measuring samples, the probe was calibrated according to the manufacturer's software (V.1.1.8). A total organic carbon (TOC) test was performed using a Total Carbon Analyzer (TOC-V CPN analyzer from Shimadzu). All water samples were filtered using a 0.2 μm cellulose filter. The concentrations of TP, TN, NH_4^+-N, NO_3^--N in the water samples were measured by a Shimadzu UV-2550 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) using a high-pressure digestion method, a molybdenum blue method, and Nessler's reagent method, respectively (Shen et al., 2014).

The overall voltage of the multi-cathode system is monitored, with the cathode electrically connected to each other. Power was calculated according to the formula, $P = iV$, where "P" = power, "i" = current, and "V" = voltage. The voltage was measured simultaneously with the external resistance of 1000 Ω. The power density (mW/m^2) was calculated as iV/A, where A is a surface area of the anode electrode (m^2), i is current (mA) and V is voltage (mV). Polarization studies were carried out after reaching a pseudo-steady state by changing the external resistances from 30,000 to 200 Ω using the resistance box (GEC 05 R Decade Resistance Box) (Jadhav et al., 2014). The Ag/AgCl reference electrode was positioned near the cathode in order to measure the cathode potential. The anode potentials were calculated by subtracting the cell potential from the cathode potential (Erable et al., 2013).

The response sensitivity of the voltage from the MS-SMFC-based sensor to DO concentration change was evaluated as artificial neural networks (Feng et al., 2013). Five main response peaks (Peak 1, Peak 2, Peak 3, Peak 4, and Peak 5) produced by the changing of DO concentrations and voltage were selected to analyze quantitative response metrics, including the peak height (PH), the peak area (PA), the acceleration rate (AR, *i.e.* rate of DO concentration or voltage increase), and the subsidence rate (SR, *i.e.*, rate of DO or voltage decrease). PH was the maximum peak height of every peak; PA was the peak area and calculated by integral method; AR and SR were calculated by linear fitting. Detailed calculation illustration was shown in Fig. S5. In addition, the sensitivity of the MFC sensor under various DO concentration scope was also defined as a change in electric signal (voltage) per unit change in the DO concentration (H.R. Jiang et al., 2017) (Eq. (2)):

$$\text{sensitivity} = \frac{\Delta V}{\Delta DO \times A} \tag{2}$$

where ΔV (mV) is the change in the voltage value, ΔDO (mg L^{-1}) is the change in the DO concentration, and A (cm^2) is the cathode surface area.

3. Results and discussion

3.1. Sensitivity of voltage output from single-cathode SMFC to DO concentration change

The correlation between DO concentration and voltage output was tested during the stable voltage generation period. At this stage, the exoelectrogenic biofilm might form on the anode and cathode, as graphite felt is suitable for the development of biocathode and bioanode in freshwater (Feng et al., 2017; Schamphelaire et al., 2010; Zhang and Angelidaki, 2012a). Even though the fuel cells were operated several times, the results were similar. Here only two representative results are presented (Fig. 2A and B): a long-term test (240 h) and a short-term test (12 h). Both of the tests showed a linear relationship between the voltage output and the DO concentration in the range of 0–9 mg L^{-1}. The correlation coefficient was high as 0.9576 (Fig. 2C). Noise was estimated according to the voltage change along different depths of water in the indoor control experiment (Fig. S6). The maximum noise value was 13.17 mV when voltage was variated underwater 40 cm. All the voltages both in the long and the short test were higher than the noise value. The detection limit was the DO concentration corresponding to the lowest voltage. In the experiment, the lowest voltage was found at 18 mV in the long-term test and the corresponding DO concentration was 0.11 mg L^{-1} (Fig. 2B). The result was comparable to the obtained by the other method reported previously (Zhang and Angelidaki, 2012b). When the DO concentrations were higher than 9 mg L^{-1}, the correlation coefficient decreased (Fig. S7).

The sensitivity of the voltage responding to the DO concentration was evaluated by the structural features, including PA, PH, AR, and SR (Fig. S8 and Fig. 2D). From the selected five peaks in Fig. 2A and B, the well response signals between voltage and DO concentration were found. It was observed that the AR and SR were smaller for Peak 1 to Peak 3 than those for Peak 4 and Peak 5, and this was due to the quicker change of DO in the short-term test. The sensitivity of the MS-SMFC sensor was much greater in DO scope of 0–2 mg L^{-1} (1.62 mV (mg L^{-1})$^{-1}$ cm^{-2}), 2–4 mg L^{-1} (1.96 mV (mg L^{-1})$^{-1}$ cm^{-2}), and 4–6 mg L^{-1} (1.90 mV (mg L^{-1})$^{-1}$ cm^{-2}) than that when DO content was higher than 6 mg L^{-1} (0.83 mV (mg L^{-1})$^{-1}$ cm^{-2}) (Fig. S9). It meant that the MS-SMFC sensor was suitable for wide scape of DO concentrations and had the ability to monitor the hypoxia condition in water column.

The above results indicate that the SMFC has the potential to be a simple and rapid DO sensor. In the experiment, the voltage output of sensor firstly needs to be maintained at high levels under the saturation of DO. In this condition, the sensor is highly sensitive to low DO concentration. So, obtaining a high voltage output is the key factor to enable the proper operation of the sensor.

3.2. Effect of multi-cathodes on voltage production from SMFC

Produced voltages from a single- and multi-cathode structure are shown in Fig. 3A. Voltage outputs were not influenced by the structure of three cathodes (Fig. S2B) at any time during the experiments. Further experiments also proved that voltage outputs were not affected by the distances between adjacent cathodes from 2 cm to 10 cm (Fig. S10). The voltage produced from the two structures increased quickly during

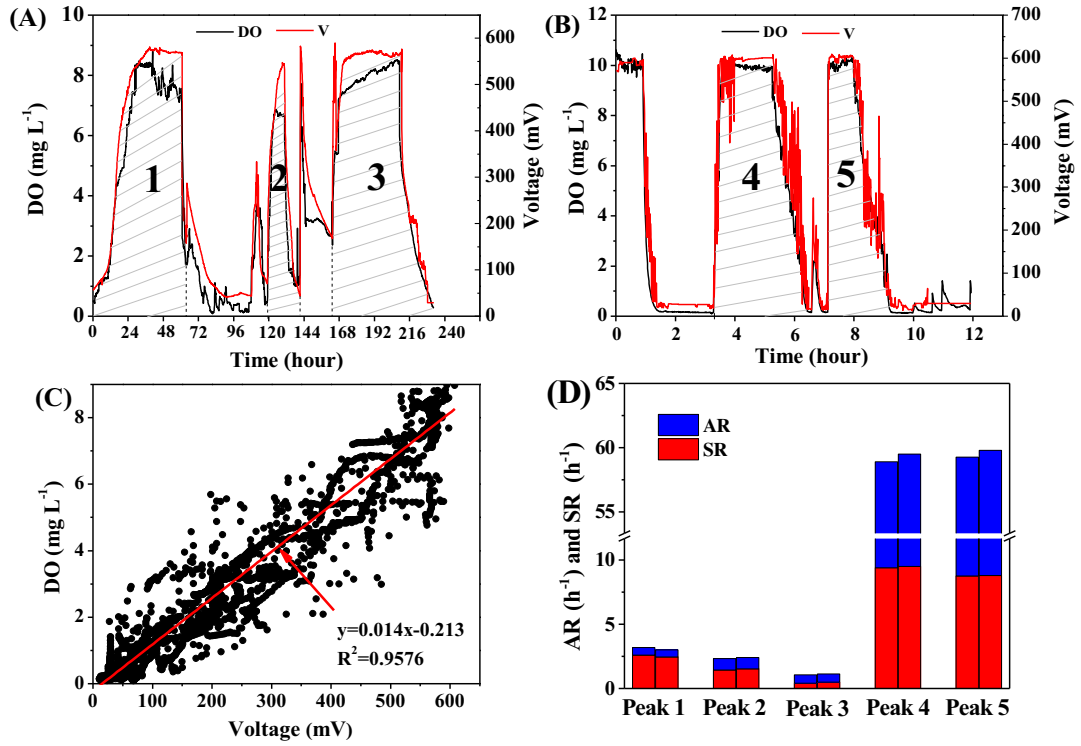

Fig. 2. Voltage generation from single-cathode SMFC and dissolved oxygen (DO) concentration in a long-term test (A) and short-term test (B), the corresponding relationship between DO (0–9 mg L^{-1}) and voltage (C), the acceleration rate (AR) and subsidence rate (SR) of voltage and DO (the left column represented the DO concentration change and the right column represented the voltage change) (D). The numbers (1–5) represented the main peaks (Peak 1, Peak 2, Peak 3, Peak 4, and Peak 5) which were produced by the changing of DO concentrations and voltage.

the initial period of 10 days. This phenomenon might be due to the formation of an electrochemically active biofilm on the anode surfaces (Zhou et al., 2014). On the 10th day of operation, both SMFC structures exhibited a peak. The single-cathode SMFCs had a slightly higher voltage (about 450 mV) than the multi-cathode SMFCs (about 400 mV), but after 60 days, the voltage for both structures was very similar.

A polarization curve represents the cell voltage as a function of the current density. The maximum achievable power densities from multi-cathode SMFCs (17.8, 16.2, and 17.1 mW m^{-2}, respectively) were just a little lower than the single-cathode SMFCs (20.1 mW m^{-2}) (Fig. 3B). From the indoor control experiment, various DO concentrations along the depths of water were obtained (Fig. 4 and Fig. S6). The voltage generation also showed the stratification phenomenon and a higher correspondence ($R^2 = 0.982$) was obtained between the voltage and actual DO concentration. The above results indicated that the structure of a multi-cathode SMFC could replicate the performance of conventional SMFCs and verified that the response from the cathodes actually was a function of a layered DO concentration.

3.3. Optimization of MS-SMFC performance for high and stable voltage generation

Several factors have been considered for restricted MFC power output while operating under field conditions, such as reactor configuration (Liu et al., 2005), electrode material and design (Hong et al., 2009), and electrode spacing (Hong et al., 2009). The series experiments were performed to optimize the key factors which affect the MS-SMFCs operation.

3.3.1. Effect of the anode to single cathode area ratio

It has been previously demonstrated that the relative sizes of the anode and cathode can affect power output (Oh and Logan, 2006). It is possible that the amount of exoelectrogenic microbes can increase with the surface. However, powers were also not affected by the surface areas of the anode and cathode (Oh and Logan, 2006), and these variations related to the structure of MFCs. In an MS-SMFC sensor, the voltages produced using the different area ratios of each anode and cathode were compared in Fig. 5. From all the different area ratios of MS-SMFC, the maximum voltage was measured around on day 7. It was found that voltage was reduced from 600 mV to 100 mV when the area ratio of anode to cathode was reduced from 11:1 to 1.8:1. This decrease is most likely due to a shortage of organic matter near the anode, required for current production, which can be attributed to limitations of the mass-transfer in the sediment (Reimers et al., 2001). Furthermore, increasing the area ratio by 14.5:1 led to a voltage decrease to 450 mV after 33 day incubation, and SMFC performance was not always improved with further increase in anode surface area. It is plausible that cathodes with less surface areas are not able to consume more electrons delivered from the anode in the sediment, causing the voltage to drop (Hong et al., 2009). This result agreed with the previous finding that the current production was limited by the cathode with a five times smaller surface area than the anode (Dentel et al., 2004). So, the optimum anode to single cathode area ratio was selected as 11:1 in the MS-SMFC sensor.

3.3.2. Effect of organic matter content in sediments

Substrates used in the microbial fuel cell (MFC) are important because they serve as carbon and energy source for the bacterial community in the anode biofilm (Sajana et al., 2014). Organic matter content in sediments affected anode potential of the cell, and also influenced voltage generation and the time of maintaining a stable voltage. Thus, effects of different contents of organic matter in sediments on voltage production were investigated. The MFCs enriched with different substrates had different acclimatization capabilities when reacting to substrate changes (Zhang et al., 2011), but how the different organic contents could influence voltage output was unknown. Fig. 6A shows time-course variations of voltages when different amounts of organic

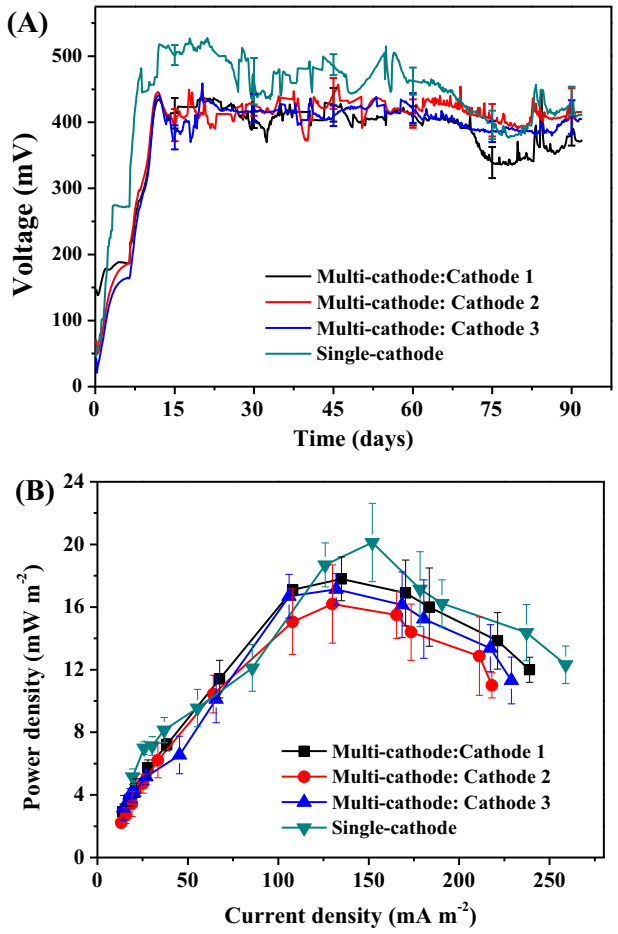

Fig. 3. Comparison of the voltage generation (A) and power densities (B) between the single-cathode and multi-cathode structure. Error bars represent the standard deviations of triplicated tests.

matter were added into sediments. All the treatments maintained very stable voltages during the whole operation period, but every performance was different. SMFC-5% (5% of organic matter content in sediments) and SMFC-4% had higher voltages outputs with peak voltages of about 600 mV and 550 mV, respectively. These voltages differed from other SMFCs, presumably due to the abundant supply of

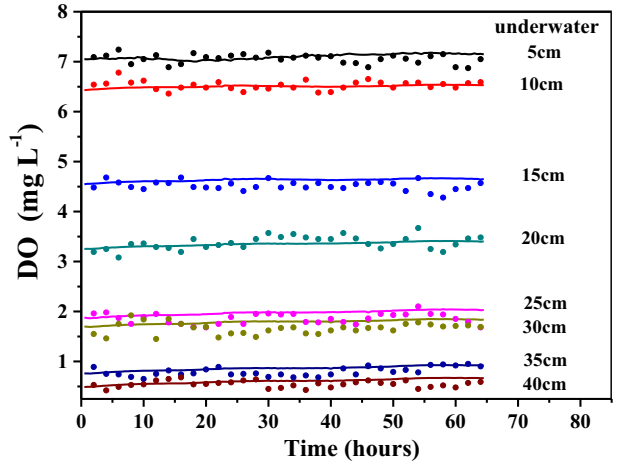

Fig. 4. DO change along different depths of water in the indoor control experiment: calculated according to the relevant equations between DO and voltage (line) and measured using DO probe (point).

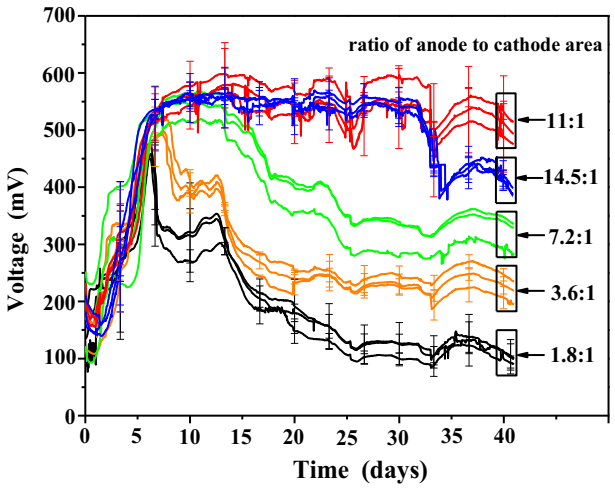

Fig. 5. Effects of ratio of anode to cathode area on the output of voltage from MS-SMFC sensor. Error bars represent the standard deviations of triplicated tests.

organic substrates. More importantly, with the increase of organic matter content, the startup time shortened, with 9 days for SMFC-5%, and 15 days for SMFC-4%, and 20 days for SMFC-2.5% and SMFC-2%. Thus, it can be reasonably explained that the maximum attainable current, including current levels at a relatively stable rate and the time needed to reach maximum current output, are dependent on the organic matter content of the sediment, which could limit the mass-

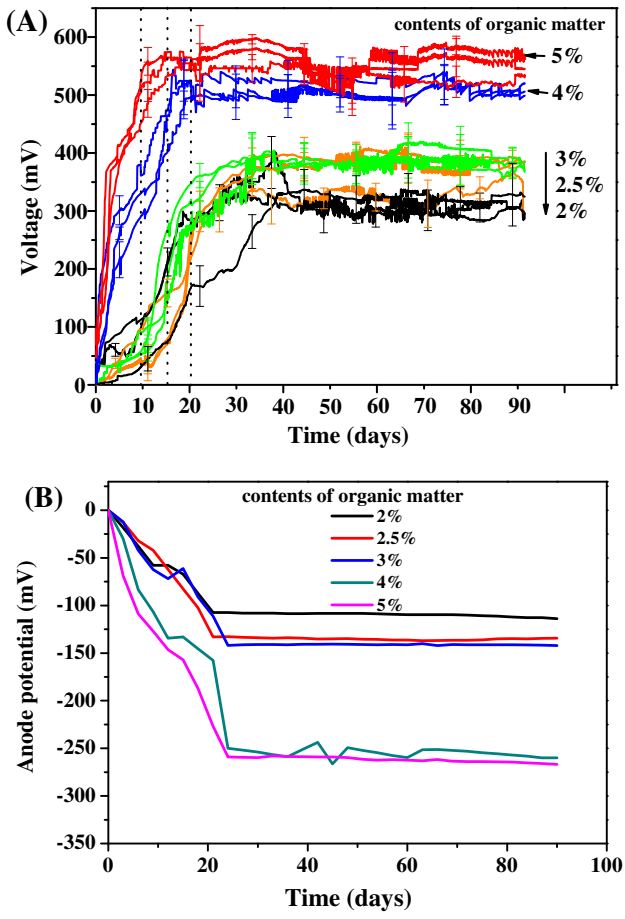

Fig. 6. Effects of organic matter contents in sediments on the output of voltage (A) and anode potential (B). Error bars represent the standard deviations of triplicated tests.

transfer in the sediment (Hong et al., 2009). As shown in Fig. 6B, SMFC-5% and SMFC-4% had higher negative anode potentials compared to those with less organic matter contents. Zhao et al. (2016) also found that appropriate organic content (loss of ignition of 5%) was important to maintain stable power outputs, while high organic content led to higher but very unstable voltage output because of biogas accumulation and worm activities and lower organic content showed low power output. It was also found that organic matter content in sediments changed the microbial community composition on the anode biofilm. This was the important reason relating to higher power outputs (Zhao et al., 2016). In fact, different anode environment mainly influenced the electricity-producing microbe (Logan and Regan, 2006; Rago et al., 2017) and their secretion (von Canstein et al., 2008), which could be used as extracellular electron shuttles. Then, the organic matter content in sediments was manipulated as 5% by adding plant residues in the other indoor experiments, in order to maintain the anode potentials and obtain the higher and more stable voltage.

3.4. In-situ application

In the *in-situ* experiments, the MS-SMFC sensor was operated for about 67 days without any servicing (*e.g.*, cleaning of electrodes or adding sediment organic matter), and the voltage showed an obvious stratification phenomenon along different depths of the water column (Fig. 7A). Voltages under water at 10 cm and 20 cm were higher during the 15–20 days, which might be in part due to the higher water temperature (Fig. 7A) (Larrosa-Guerrero et al., 2010). DO concentrations were measured using a DO meter every two weeks during the *in situ* experiments, and also measured every hour on day 30. The linear relationship between voltage and the actual DO concentration was observed with linear regression coefficients of 0.8897 (Fig. 7B). According to the linear relationship, DO concentrations at various depths of the water column were calculated (Fig. 7C) and the phenomenon of DO stratification in a shallow lake was found. DO concentration at the surface of water (0–10 cm) was about 3–4 mg L^{-1}, and in the range of 1–3 mg L^{-1} at the water depth of 10–20 cm. Especially, hypoxia phenomenon was observed at the water depths of 20–30 cm and 30–40 cm, with DO concentration less than 0.5 mg L^{-1} (Fig. 7C). In order to determine whether the sensor could monitor the change in the DO throughout the whole day, DO concentrations on day 30 were further measured by DO meter and also calculated from voltage of MS-SMFC sensor (Fig. 7D). The DO concentration was lower during night than during daytime, with the lowest DO concentration (about 0.2 mg L^{-1}) from 3 to 6 o'clock in the morning and the highest DO concentration (about 4.0 mg L^{-1}) from 13 to 14 o'clock in the afternoon. The DO variation may be caused by the respiration difference (Hanson et al., 2008). Enhanced respiration but weakened photosynthesis of phytoplankton in lake water leads to the minimum value of DO in the morning. However, enhanced photosynthesis results in the maximum content of DO in the afternoon. Compared to the actual DO concentration measured using the DO meter, similar changing trends were found on Day 30 (Fig. 7D), which indicated that the sensor could well monitor the DO concentration in the actual lake water. Higher TOC content and lower DO content (Table S1) were found in the lake water than observed in other bays of Taihu Lake (Zhao et al., 2017) and other lakes (Xu and Guo, 2017). In fact, the experimental site was in the macrophyte-dominated zone of Taihu Lake. Excess nutrients and organic matter remained in the lake water after macrophyte detritus degradation, which led to a depletion of DO along the depth of water column. Also, organic matter content in sediments of the test site was as high as 4.5%. So, the voltage was not limited by organic matter content in this *in-situ* experiment. It is interesting to note that there was only a little difference between the linear relationships of the voltage and the DO in both the indoor and *in-situ* experiments. So, it seemed that the MS-SMFC sensor for

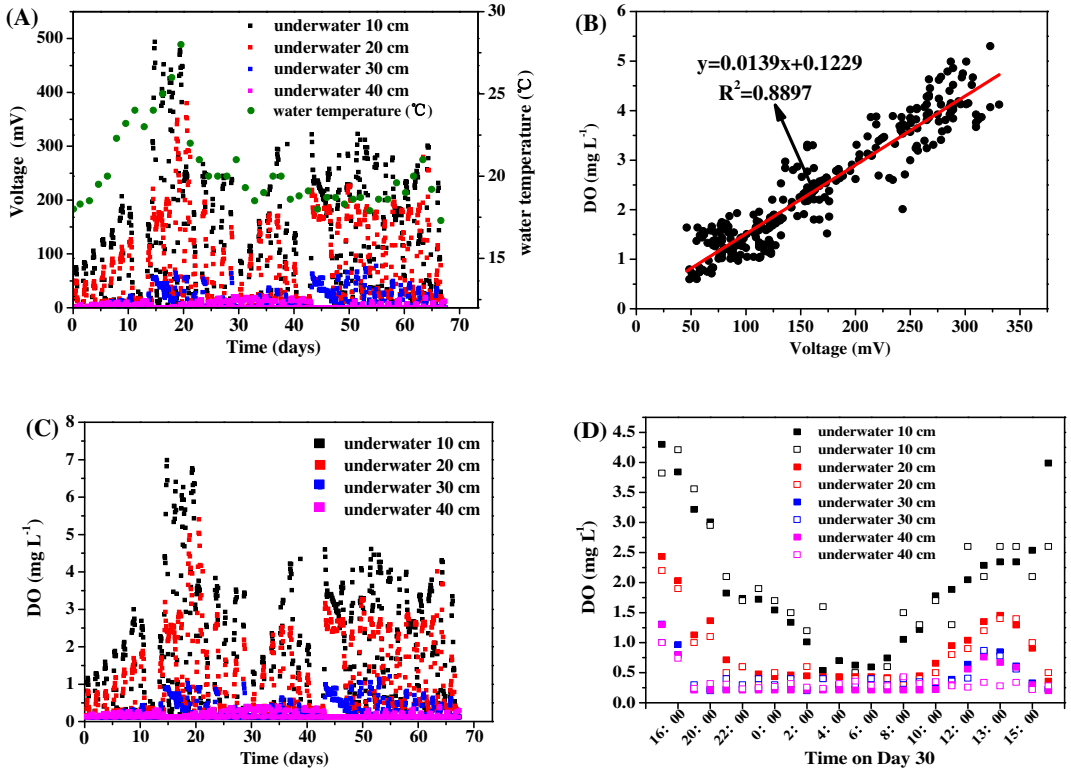

Fig. 7. Voltage output from MS-SMFC sensor during the 67 days of *in-situ* experiment (A), corresponding relations fitted using DO data measured with the DO meter and the voltage output with an MS-SMFC sensor (B), DO concentrations calculated according to the corresponding relations (C); and change of DO concentrations measured using MS-SMFC sensor (solid point) and DO meter (hollow point) on Day 30 (D).

DO measurement might be unaffected by environmental conditions. In fact, the MS-SMFC sensor could be tested first to get the linear relationship before monitoring the DO concentration in the practical application.

3.5. Application prospect

The above results indicate that the MS-SMFC biosensor exhibited many improvements and compared favorably to other conventional DO sensors or measurement methods. First, compared with the existing MFC-based DO sensors (Velasquez-Orta et al., 2017; Zhang and Angelidaki, 2012b), the MS-SMFC biosensor can realize the simultaneous online monitoring of DO concentration at different depths of water by employing only one set of MS-SMFC system with the multi-cathode single-anode configuration, not multiple-unit MFC systems. Second, from the economic consideration, this type of sensor cost is less expensive than conventional DO meter when simultaneously monitor DO concentrations along different depths of lake water. Although the sensor needs more preparation before measuring, it does not need any servicing during the long-term operation. So, compared to the traditional DO meter, it could be used as a cheap online monitoring system. Third, as the SMFC-biosensor had a quick response to the change of DO in water environments, so it could be applied of forecasting the sudden pollution of aqueous environment, such as the dead zone risk in a shallow lake. Forth, the biosensor system remains stable and can adapt to different conditions, as it will not be affected by the nutrient concentrations in lake water (Table S2 and Fig. S11). Although the concentration of NO_3^--N used in this study was not high, a previous study determined that the maximum voltage output was not affected by the nitrate at higher external resistance (270–1000 Ω), but possibly due to the low organic carbon availability (Sukkasem et al., 2008). From the above, the MS-SMFC system has the potential to be integrated well with wastewater treatment, biosensing and real-time online monitoring.

Nevertheless, further research is planned as we improve the online real time MS-SMFC biosensor. Considering the effects of organic matter content in sediments on the performance of sensor, we are designing an improved sensor which will include a container. Sediments which contained 5% contents of organic matter were put into the container. Then anode of sensor was placed in the sediments. So, the operation of the sensor will not influenced by the organic content. The integrated system can also be validated to illustrate the general application of this biosensor in more lakes after investigating effects of other factors, such as long electrode distance and short startup time.

4. Conclusions

A novel multi-cathode, single-anode system integrating a sediment microbial fuel cell-based biosensor was developed. Stable and continuous voltage production after the appearance of the peak value was the key factor in sensor performance. This could be obtained under the condition of 11:1 of anode to single cathode area ratio and 5% organic matter content in sediment. Due to the special configuration of the MS-SMFC sensor, it could realize *in situ*, continuous, and online monitoring of the DO concentration at different depths of lake water and could be used as an early-warning system to help identify environmental risks. Still, further works are expected before using the MS-SMFC sensor, such as, the time of stable voltage generation should be shortened, and finally realized the more convenient operation in environment water.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.04.032.

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