

A simple and rapid method for monitoring dissolved oxygen in water with a submersible microbial fuel cell (SBMFC)

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ABSTRACT

A submersible microbial fuel cell (SBMFC) was developed as a biosensor for *in situ* and real time monitoring of dissolved oxygen (DO) in environmental waters. Domestic wastewater was utilized as a sole fuel for powering the sensor. The sensor performance was firstly examined with tap water at varying DO levels. With an external resistance of 1000 Ω , the current density produced by the sensor (5.6 ± 0.5 – 462.2 ± 0.5 mA/m²) increased linearly with DO level up to 8.8 ± 0.3 mg/L (regression coefficient, $R^2=0.9912$), while the maximum response time for each measurement was less than 4 min. The current density showed different response to DO levels when different external resistances were applied, but a linear relationship was always observed. Investigation of the sensor performance at different substrate concentrations indicates that the organic matter contained in the domestic wastewater was sufficient to power the sensing activities. The sensor ability was further explored under different environmental conditions (e.g. pH, temperature, conductivity, and alternative electron acceptor), and the results indicated that a calibration would be required before field application. Lastly, the sensor was tested with different environmental waters and the results showed no significant difference ($p > 0.05$) with that measured by DO meter. The simple, compact SBMFC sensor showed promising potential for direct, inexpensive and rapid DO monitoring in various environmental waters.

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1. Introduction

Oxygen is involved in various biological, chemical and biochemical processes in environmental waters. Dissolved oxygen (DO) is probably one of the most important and frequently used parameters related to water quality (Wetzel, 2001). The DO levels provide vital information regarding the biological and biochemical reactions in aquatic environments. For example, DO can be used to measure the capacity of biological processes that take place in environmental water sites, by which organic matter can be decomposed (Markfort and Hondzo, 2009). DO measurement is also the basis of various water quality tests such as the 5-day biochemical oxygen demand measurement (BOD) (Chen et al., 2007). Besides, DO is an essential indicator of microbial metabolism in biological wastewater treatment process due to its important role in biochemical reactions (Ansa-Asare et al., 2000).

A significant number of chemical, physical and electrochemical methods have been established to measure DO in water (Chen et al., 2007; Levy et al., 1977; Sakai et al., 2004). Among them, electrochemical methods are widely employed because of its simplicity and high sensitivity. Various electrodes and microelectrodes have

been developed and regarded as the most common electrochemical method for DO measurement (Bencsik et al., 2010; Ju and Nallagatla, 2003; Luz et al., 2006). However, these methods still have drawbacks, such as expensive electrode materials (e.g., gold), time consuming, easily poisoned, membrane fouling and not readily for long-term *in situ*, online or remote monitoring. Moreover, most of the commercial DO sensors are powered by external power sources or battery, which adds on the operation and maintenance cost, especially for long-term monitoring activities. Therefore, reducing construction and energy cost are critical for the development of efficient and cost-effective DO sensors. An alternative strategy is direct measurement of DO with microbial fuel cells (MFCs).

MFCs are bioelectrochemical reactors in which microorganisms oxidize organic matter and produce electrons at the anode, while terminal electron acceptors (e.g. O₂) are reduced by accepting these electrons at the cathode (He et al., 2007; Logan and Regan, 2006; Rabaey and Verstraete, 2005; Zhang et al., 2011). Regarding the anodic oxidation, current generation is dependent on the microbial respiration and proportional to the fuel concentration, therefore, MFCs have been widely tested as BOD, microbial activity and toxicity sensors (Chang et al., 2004; Davila et al., 2010; Stein et al., 2010; Williams et al., 2009; Zhang and Angelidaki, 2011). However, another possible application of MFCs has not been explored, which is DO monitoring. In theory, number of electrons transferred to anode by microbes, should

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correspond to electrons reacting with oxygen at cathode, and thus, the current generated could also be a measure of DO concentration. Besides easy measurement, using MFCs as DO sensor has another important advantage of not requiring an external power source. Various organic wastes and sources of wastewater have been used as fuel for power generation in MFCs (Pant et al., 2010; Zhang et al., 2009; Zhang and Angelidaki, 2012a). Therefore, MFCs have potential to be a direct, quantitative and real time DO sensor.

In this study, a submersible MFC (SBMFC) was tested as a DO sensor. The SBMFC has only an anode chamber, which makes it applicable for *in-situ* monitoring of various environmental waters. Most importantly, wastewater and cheap carbon paper were used as fuel and electrode materials, respectively. The aim was to develop a more compact and simple system. The performance of the SBMFC sensor was evaluated in terms of DO detection range, response time and reproducibility. The effect of different operation parameters (e.g., substrate, temperature, pH, conductivity, nitrate level, and external resistance) on the sensor performance was also investigated. The development of SBMFC sensor will provide an advantageous DO sensor design and expand the application of MFCs technology.

2. Material and methods

2.1. Environmental water samples

Domestic wastewater was collected from primary clarifier (Lundtofte Wastewater Treatment Plant, Lyngby, Denmark), which was used as fuel and inoculum in the SBMFC. The chemical oxygen demand (COD) concentration of the wastewater was 300 ± 20 mg/L. Sea water was collected from North Sea at Bellevue, Denmark ($55^{\circ}46' N$, $12^{\circ}35' E$). The lake sample was taken from the Bagsvaerd Lake ($55^{\circ}46' N$, $12^{\circ}27' E$) in Denmark. The bioreactor sample was taken from a 2 L algae cultivation reactor (*Chlorella vulgaris*, 3 g/L). No pretreatment was performed prior to analysis for all the samples.

2.2. SBMFC construction and operation

The SBMFC, which was made of nonconductive polycarbonate plates, was a rectangular reactor composed of only one chamber, i.e. the anode chamber (inside dimensions $3 \text{ cm} \times 3 \text{ cm} \times 2 \text{ cm}$) (Fig. 1). A sandwich structured membrane electrode assembly (MEA) was placed at the end of anode chamber (cathode electrode facing outside). The whole reactor was assembled with twelve stainless screws and sealed with rubber gaskets to prevent leakage (Fig.1). The MEA was prepared by hot-pressing the anode electrode, proton exchange membrane (Nafion 117, DuPont Co., USA) and cathode electrode together (Min and Logan, 2004). Both the anode and cathode electrode were made of non wet-proofed carbon paper ($3 \times 3 \text{ cm}^2$, Toray carbon paper, E-TEK division, USA). Plastic tubes were connected with the anode chamber for fuel refill. Electrical connections and electrode pretreatment were done as previously described (Zhang and Angelidaki, 2012b).

To start up the SBMFC and enrich electrochemically active biofilm on the anode, the SBMFC was firstly operated for about two and half months. During the enrichment period, the SBMFC was submersed 2 cm below the water surface in a glass reactor (total volume of 1000 mL) which was fed with 500 mL of tap water (pH 7.3). 18 mL of the domestic wastewater (300 ± 10 mg COD/L, pH 7.2) was fed into the anode chamber as both inoculum and fuel. The anode chamber was purged with nitrogen gas for 10 min to remove oxygen before operation. The DO level in the glass reactor was continuously controlled at 8.8 ± 0.5 mg/L with a standard gas (21% oxygen). The SBMFC was connected to an external resistance of 1000Ω . The wastewater in the anode chamber and tap water in the glass reactor were refilled respectively when the voltage is lower than 20 mV. After two and half months enrichment, stable and repeatable current density was produced, indicating that the reactor was ready for the test. During the test period, the SBMFC was submersed in the same glass reactor used in the enrichment period and operated in batch mode. The tap water was modified according to different purposes (e.g., different pH and conductivity) to evaluate the sensor performance and afterwards the sensor was validated with real

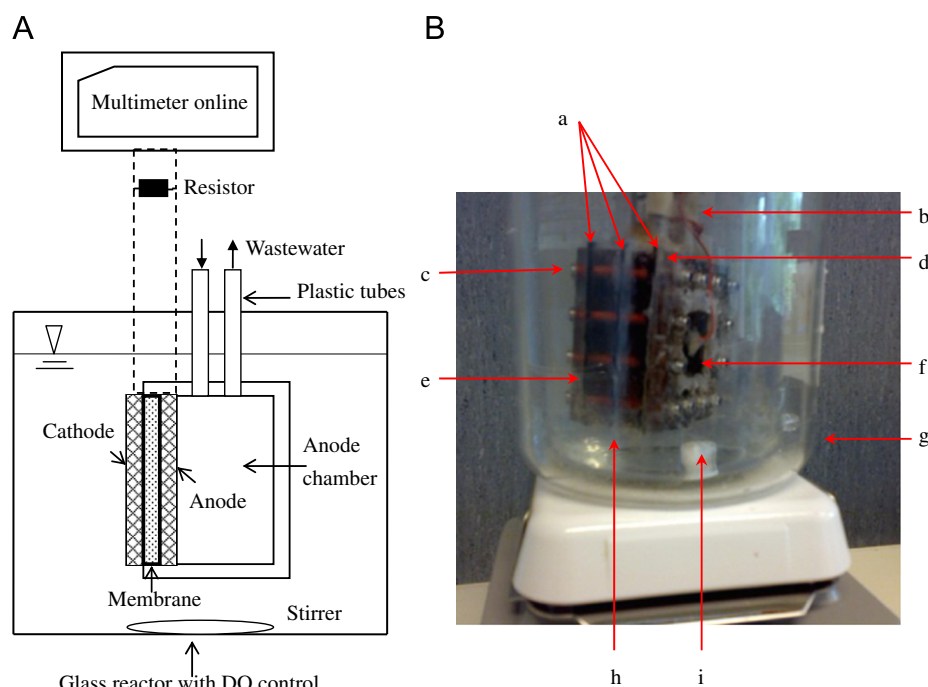


Fig. 1. Schematic diagram (A) and image (B) of the SBMFC sensor. Labeled items in (B): a-rubber gasket; b-plastic tube; c- screw; d-cover plate (polycarbonate); e-cover plate; f-cathode side of MEA (anode and membrane cannot be seen from the current view); g-glass reactor; h-anode chamber; and i-stirrer.

environmental water samples. Unless otherwise stated, the glass reactor was operated at room temperature (20 ± 5 °C). For testing the sensor with different anodic fuel concentrations, the domestic wastewater was diluted with deionized (DI) water or amended with acetate to reach COD concentration of 100–1000 mg/L. The pH of all the solutions was adjusted with 1 M HCl or NaOH solutions. The conductivity was adjusted with KCl (0.1 M) solution. All electrode transfers, inoculation procedures and wastewater replacement were conducted in an anaerobic glove box (Coy Laboratory Products Inc.). Nitrogen gas and standard gases containing either 100% or 21% oxygen were used to control the DO level in the glass reactor throughout the tests. During operation, no precautions were taken to maintain anaerobic conditions in the anode chamber. All experiments were carried out in duplicate.

2.3. Electrochemical analyses and calculations

COD was analyzed according to the standard method (APHA et al., 1995). Conductivity was determined with a CDM 83 conductivity meter (Radiometer), while pH was measured with a PHM 210 pH meter (Radiometer). Nitrate was quantified by ion chromatography (Dionex DX-300 fitted with an AS-9-HC column and conductivity detector, Dionex Co., Sunnyvale, CA, USA). DO was measured using a DO meter (Microprocessor oximeter 539, Germany).

The voltage (V) across an external resistor in the electrical circuit was monitored with 2 min intervals using a multimeter (Model 2700, Keithley Instruments, Inc., Cleveland, OH, USA) linked to a differential multiplexer (Model 7701, Keithley Instruments, Inc.). The current intensity (I) was calculated according to Ohm's law, $I = V/R$, where V is the voltage and R is the resistance. Current density (J) was calculated as $J = I/A$, where A is the projected surface area of the anode electrode.

3. Results and discussion

3.1. The response of current generation to different DO levels in tap water

The SBMFC sensor was tested in tap water with an external resistance of 1000 Ω . Fig. 2A shows the dynamic response of the sensor to different DO levels. The current density increased stepwise as the DO level increased from 0 to 8.8 ± 0.3 mg/L. After each change of DO level, a maximum response time of 4 min was observed for the sensor to reach a new steady-state current (Fig. 2A), indicating the good sensor sensitivity. The correlation between current density and DO level was established as shown in Fig. 2B. It is clear shown that the current density (5.6 ± 0.5 – 462.2 ± 0.5 mA/m²) increased linearly (regression coefficient, $R^2 = 0.9912$) with DO up to 8.8 ± 0.3 mg/L. Nevertheless, the increase was not significant by further increasing DO concentration, which suggested that the current generation was nearly saturated. The SBMFC also presented a good reproducibility for oxygen determinations. The relative standard deviation of the current density at each DO level was less than 5% (Fig. 2B). The detection limit, response time and reproducibility of the SBMFC sensor observed here were comparable to those obtained by other methods (e.g. chronoamperometry) reported previously (Chen et al., 2007; Ribeiro et al., 2004). Luz et al. (2006) reported a detection limit of 0.2–8.0 mg-O₂/L and relative standard deviation of 4.5% by using differential pulse voltammetry and chronoamperometry method, which was similar to the results observed in this study. The above results indicate that the SBMFC has the potential to be a simple and rapid DO sensor. Compared with conventional DO sensors or measurement methods, the SBMFC has its own advantages. Firstly, there is no need of external power sources. Normally, a commercial DO meter with working voltage of 230 V and working current of 10 mA needs about

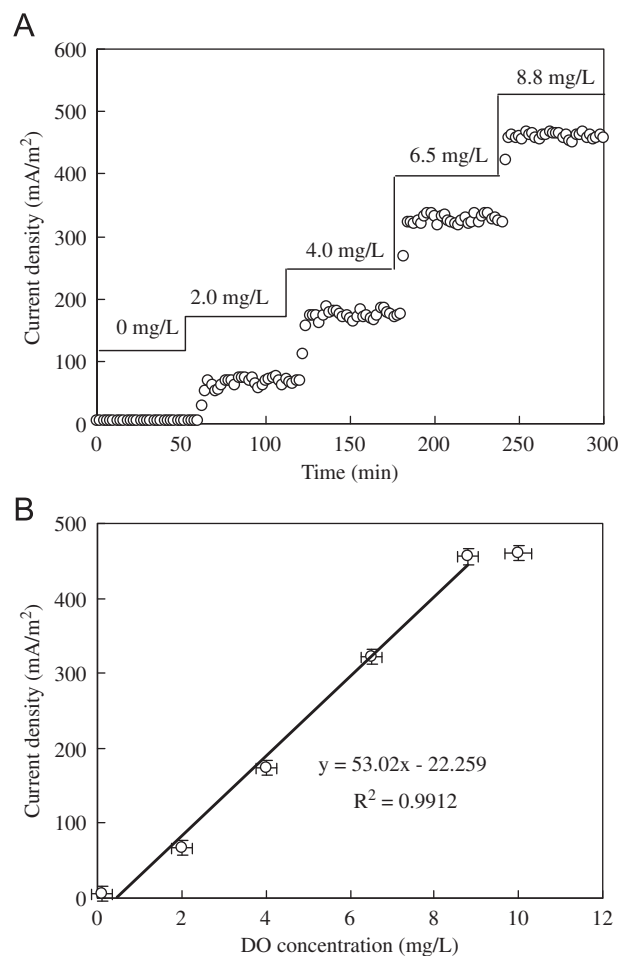


Fig. 2. Time course of current generation at different DO levels (A) and relationship between steady-state current density and DO concentration (B). The step increasing line with numbers (0, 2.0, 4.0, 6.5, and 8.8 mg/L) in the (A) indicates the DO level controlled in the glass reactor during the test period. Operation condition: temperature, 20 °C; pH, 7.3; conductivity, 500 μ S/cm; and fuel concentration, 300 mg-COD/L.

20 kw h electric energy for one year operation, not to mention the cost for electricity transmission in remote area. The sole fuel to power the SBMFC is wastewater, which makes the sensor suitable for long-term monitoring, especially in the field of biological wastewater treatment. Secondly, the electrodes used in the SBMFC sensor are carbon paper without any modification, which are stable and much cheaper than the expensive metals (e.g., gold, silver) used in electrochemical sensors. Thirdly, the electrical current as a direct, real time measure of DO can be easily measured. The sensor can be easily connected with various data logging system for online monitoring. Lastly, due to its submersible configuration, the SBMFC can be applied *in situ* to the existing reactors or natural environmental waters without extra construction. The SBMFC was operated for over 6 months with stable output, without any servicing (e.g., cleaning of electrodes). Further optimization of the electrode material and surface area, microbial efficiency, mass transfer, or cathode design could further enhance the sensor performance. In addition, recent development in micro-MFCs (Qian et al., 2009; Qian et al., 2011) provided an opportunity to widen the application niches of the SBMFC sensor, e.g., in biofilms, aerobic granules.

3.2. Sensor performance with different external resistances

It has been previously demonstrated that external resistance can affect the electricity generation of MFCs (Aelterman et al., 2008; Pinto

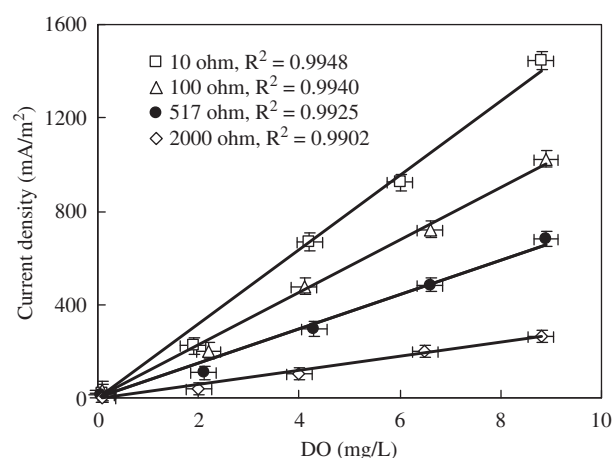


Fig. 3. Effect of external resistance on DO measurement. Operation condition: temperature, 20 °C; pH, 7.3; conductivity, 500 μ S/cm; fuel concentration, 300 mg-COD/L.

et al., 2010). A series of tests was therefore performed in which the external resistance was varied from 10 to 2000 Ω to explore the effect of external resistance on the sensor performance. As shown in Fig. 3, a linear relationship between current density and DO concentration was observed for all the tested external resistances. The linear regression coefficient was higher than 0.9902, in all the external resistance of 2000, 517, 100, and 10 Ω , tested. Additionally, the linear regression coefficient was slightly increased with external resistance. It was also observed that the difference among the current densities obtained with different external resistances was much bigger at high DO level than that at low DO level (Fig. 3). For example, at DO level of 8.8 ± 0.3 mg/L, the current density generated with an external resistance of 10 Ω was 1444 ± 5 mA/m², which was much higher than that obtained with 2000 Ω (266 ± 5 mA/m²). However, at DO level of 2 ± 0.3 mg/L, the difference between the current densities was not as big as observed above. It could be due to that the external resistance was the main limiting factor for current generation at high DO levels (e.g. 8.8 ± 0.3 mg/L in this study), while the DO concentration turned to be the main limiting factor at low oxygen levels (e.g. 2 ± 0.3 mg/L) at cathode (Fu et al., 2011). The results indicate that the external resistance is important for the sensitivity of the sensor. The following issues need to be taken into account for choosing an appropriate external resistance. The first one is with size of external resistance for detection of measurable current and the other issue is the oxygen consumption by the sensor itself during monitoring. According to Ohm's Law, the lower the external resistance the higher the current, and thus, the higher oxygen would be consumed at the cathode (Aelterman et al., 2008; Pham et al., 2004). As an example the sensor would be able to operate continuously for 8.72 h, at the maximum current density (1444 ± 5 mA/m²) generated with an external resistance of 10 Ω and DO level of 8.8 ± 0.3 mg/L, to consume 1 mg oxygen (12.06 C electrons). With an external resistance of 2000 Ω , this period will be 5 times longer. Therefore, using of relative higher external resistance could lower the impact of oxygen consumption on the monitoring activities. Considering the above, 1000 Ω was used in the following experiments.

3.3. Effect of anodic substrate concentration on the sensor performance

Substrate concentration is a critical parameter for microbial electricity generation. In order to investigate its effect on the sensor performance, the modified domestic wastewater with different COD concentrations (100, 300, 500, 1000 mg/L) was fed into the SBMFC. Fig. 4 shows the correlation between current density and DO

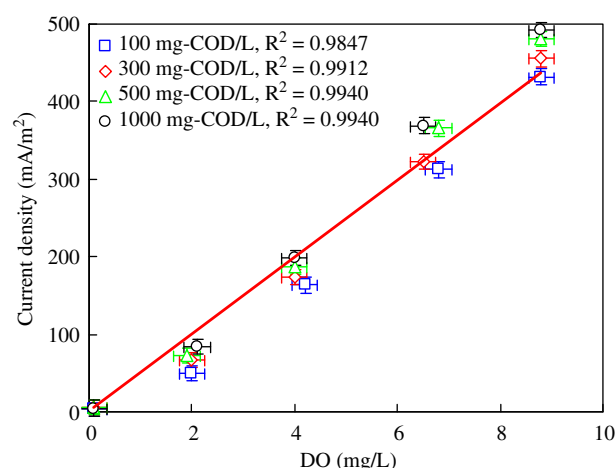


Fig. 4. Effect of anodic substrate concentration on DO monitoring. Operation condition: temperature, 20 °C; pH, 7.3; conductivity, 500 μ S/cm; and external resistance, 1000 Ω .

concentration at different substrate concentrations. A linear relationship between current density ($0\text{--}373 \pm 0.5$ mA/m²) and DO concentration ($0\text{--}8.8 \pm 0.3$ mg/L) was observed at all the tested substrate concentrations. It was found that the higher substrate concentration resulted in slightly higher slope, but the difference was not visible, which indicated that the organic matter content in the domestic wastewater was sufficient to power the sensor. In addition, the difference between the current densities generated with different substrate concentrations was much larger at high DO level than that observed at low DO level, which indicated that the fuel oxidation was the main limiting factor at high DO levels. Overall, the results indicate that the sensor can use domestic wastewater as a fuel for current generation, which would greatly reduce the operation and maintenance cost.

3.4. Effect of environmental parameters on the sensor performance

Temperature, pH, conductivity, as well as alternative electron acceptor in the water can influence the electrocatalytic oxygen reduction reaction at the cathode (Zhao et al., 2006). Thus, the effect of these environmental parameters on the sensor performance was investigated. The influence of the pH (5.5, 7.3, 8.0 and 9.0) on the sensor performance is shown in Fig. 5A. A linear relationship between current density and DO level was observed at all the pH tested, but the corresponding linear regression coefficients were different. At a lower pH a higher linear regression coefficient was observed, e.g., the highest value was 0.9929 at pH 5.5. It was also found that at a lower pH a higher current density was produced at a certain DO level (Fig. 5A), which could be due to the improved oxygen reduction efficiency at the cathode by increasing the acidity of the catholyte (Biffinger et al., 2008). Similarly, a linear relationship between current density and DO level was observed at different temperatures (Fig. 5B). There was no much difference between the current densities generated at different temperatures at a certain DO level, suggested that the sensor could work at an ambient temperature with less deviations. The current density also showed linear relationship with DO concentration in the waters with different conductivity (Fig. 5C). It is clear that the conductivity has less influence on the current density when the conductivity was higher than 200 μ S/cm. The effect of the presence of nitrate in the water on the sensor performance was also investigated. As shown in Fig. 5D, when the DO level was higher than 2.0 ± 0.3 mg/L, the current density was not affected by the presence of nitrate (2.5, 5 and 10 mg-N/L). However, when the DO level was lower than 2 ± 0.3 mg/L, the nitrate presence at high concentrations resulted in great deviations on DO measurement.

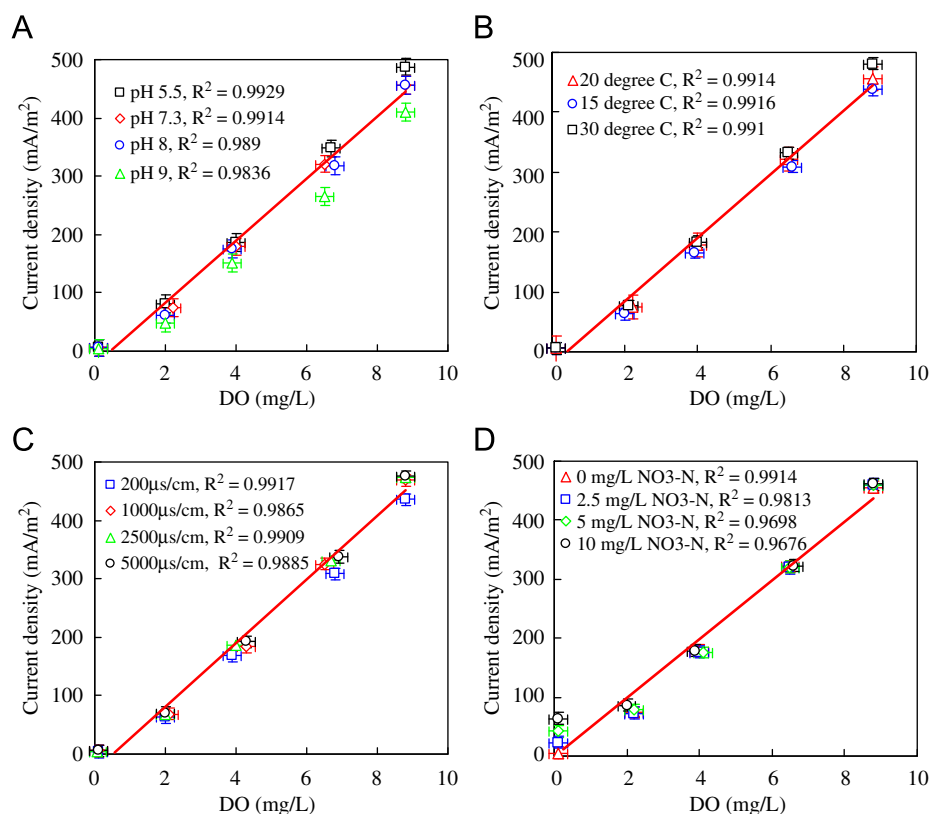


Fig. 5. The effect of water pH (A), temperature (B), conductivity (C) and nitrate content (D) on sensor performance. Operation condition: temperature, 20 °C (not applicable for (B)); pH, 7.3 (not applicable for (A)); external resistance, 1000 Ω ; conductivity, 600 $\mu\text{S}/\text{cm}$ (not applicable for (C)); and fuel concentration, 300 mg-COD/L.

Table 1

Determination of DO in lake, sea, wastewater, bioreactor and tap waters by the SBMFC sensor and DO meter.

Sample	pH	Temperature (°C)	Conductivity ($\mu\text{S}/\text{cm}$)	Nitrate (mg-N/L)	Dissolved oxygen (mg O_2/L)	
					SBMFC sensor	DO meter
Seawater	7.8 ± 0.1	20 ± 1	50130 ± 10	– ^a	6.7 ± 0.3	7.5 ± 0.2
Wastewater	6.9 ± 0.1	18 ± 1	1305 ± 5	1.5 ± 0.2	2.4 ± 0.4	2.8 ± 0.1
Tap waters	7.3 ± 0.1	20 ± 1	430 ± 5	–	7.3 ± 0.1	7.2 ± 0.1
Lake	6.6 ± 0.1	19 ± 1	1200 ± 5	1.1 ± 0.1	5.4 ± 0.5	6.1 ± 0.1
Bioreactor	8.1 ± 0.1	20 ± 1	1386 ± 5	15.8 ± 0.1	8.6 ± 0.3	8.1 ± 0.1

^a Under detection limit.

The linear regression coefficient was only 0.93 at nitrate concentration of 10 mg-N/L. Nitrate is a prevalent electron acceptor in aquifer environments (Chang et al., 2005; Fang et al., 2011). At low DO level, nitrate can serve as an electron acceptor at cathode, while the reduction of nitrate at cathode was not obvious when the oxygen was sufficient (Virdis et al., 2010), which could explain for above observation. Besides above parameters, the absorption of chemicals from the monitored waters on the cathode may affect the long-term stability of the sensor, which could be the subject of further study. These results underline that the sensor needs calibration by taking environmental conditions into account before field application.

3.5. Application to the environmental waters

The SBMFC sensor was applied to measure DO levels in seawater, wastewater, lake, bioreactor samples and tap waters, accompanied with DO measurement with a DO meter. The results are summarized in Table 1. The results obtained with the SBMFC sensor were consistent with those measured with DO meter. T-test was performed

to evaluate the data for these two methods. At 95% confidence level, there was no significant difference between the results obtained with these two methods, demonstrating the accuracy of the SBMFC sensor. Nevertheless, a slightly deviation between the DO levels measured by the SBMFC sensor and DO meter still existed, which might have been due to the influence of some characteristics of the monitored waters (e.g., pH, temperature, conductivity, alternative electron acceptors). The response time of the sensor was less than 2 min, which was comparable to that of the DO meter. The above results indicate the applicability of the SBMFC sensor in environmental waters. Due to the special configuration and small size of the SBMFC sensor, it could be taken out conveniently for *in situ* monitoring in the field, especially for the long-term monitoring in the remote area where normal DO meter would consume more energy for signal transfer.

4. Conclusion

A simple and accurate SBMFC sensor able to monitor rapidly DO level in various environmental waters was successfully developed.

Results presented demonstrated a reproducible microbial mediated current generation as a function of DO concentration. The external resistance and anodic substrate concentration affected the current density, which therefore resulted in different linear regression coefficients. The sensor performance was also influenced by environmental conditions of the monitored waters, including temperature, pH, conductivity and alternative electron acceptors. This work introduces the concept of using MFC technology for DO monitoring. Furthermore, the electricity produced from monitoring activities having potential to be further utilized by other low power sensors (e.g., salinity sensors, telemetry systems) with appropriate engineering and controls on the operational environment, which would expand the application of MFC-based biosensor. The sensitivity, response time and detection limit of the sensor can certainly be further improved in accompany with the development of MFCs technology. The SBMFC sensor provides a new way for *in situ* and quantitative monitoring of DO in a variety of environmental waters.

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