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RESEARCH ARTICLE

Biodegradability Assessment of Wastewater: A Novel Microbial Fuel Cell-based BOD_Q Sensor

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ABSTRACT: Wastewater treatment can effectively alleviate the global water crisis. Currently, biological technology is predominantly employed in wastewater treatment, with the five-day biochemical oxygen demand (BOD5) serving as a key indicator for evaluating the efficiency of biological treatment processes. However, conventional BOD5 detection methods are time-consuming and fail to provide prompt feedback to wastewater treatment plants. This study developed a microbial fuel cell (MFC)-based sensor to enable rapid detection of BODQ. Herein, four types of external resistance (1000 Ω, 500 Ω, 100 Ω, 10 Ω) were compared to evaluate the sensitivity and linearity of MFC sensors. The result showed that using the 100Ω resistor provided both the highest sensitivity (K=0.197) and linearity (R2=0.993), proving to be optimal. On this basis, key points in the change of current overtime (peak current, fastest decay, and minimum current) were analyzed to identify the detection time that provides the best balance between accuracy and speed. It was demonstrated that using the current of fastest decay as the observation point provided the optimal balance among sensitivity, linearity, and detection time. By combining these optimizations, the practicality of the MFC-based BODQ sensor was significantly improved, reducing detection time from one day to several hours while maintaining data consistency. This discovery represented an important step toward developing real-time wastewater quality monitoring tools essential for environmental management.

KEYWORDS: Environmental Sciences, Wastewater Treatment, Microbial Fuel Cell Sensor, Biochemical Oxygen Demand, Biodegradability Assessment.

Introduction

Water pollution remains one of the most pressing environmental issues of the 21st century, threatening not only the stability of ecosystems but also directly impacting human health and sustainable development. As urbanization and industrial activities expand, the discharge of organic pollutants into rivers, lakes, and oceans continues to increase. Wastewater treatment plants (WWTPs), therefore, play a vital role in mitigating the effects of such contamination. These plants rely heavily on biological treatment technologies, in which the biodegradability of organic matter, often quantified as biochemical oxygen demand (BOD), is a crucial indicator for assessing wastewater biological treatment efficiency.[1]

At present, the five-day BOD (BOD_5) test remains the international global standard for evaluating the biodegradability of wastewaters.[2] BOD_5 measures the amount of dissolved oxygen consumed by microorganisms over five days as they metabolize organic matter under controlled conditions. While this method is scientifically well established, it is impractical for rapid feedback and adjustments. In practice, WWTP operators cannot afford to wait five days for results when immediate adjustments to treatment processes are required.[3] Hence, the development of rapid, reliable, and field-deployable alternatives to BOD_5 has become an urgent research priority worldwide.[4]

In recent years, microbial fuel cell (MFC) based sensors have attracted growing attention as a promising solution to this challenge.[5] MFC is a bioelectrochemical device that is capable of directly oxidizing organic matter in wastewater to generate bioelectricity through microbial intracellular catalytic activity.[6-8] The generated electricity signal is directly proportional to organic concentration, thus MFCs can serve as sensitive indicators of BOD (named as BOD_Q) in wastewater.4 While BOD_5 measures biological oxygen depletion in wastewater over 5 days, BOD_Q measures the total coulomb generated by microorganisms as they degrade organic matter. Quantitatively, this accumulated charge is stoichiometrically converted to an oxygen equivalent (mg O_2/L), allowing BOD_Q to serve as a rapid, highly correlated proxy for standard BOD_5 values. Unlike the BOD_5 test, which requires 5 days to detect BOD values, MFC sensors can estimate BOD within approximately one day.[9-12] In conclusion, MFC-based BOD_Q sensors have a significant advantage in evaluating the biodegradability of wastewater.

Accuracy and detection time are two key factors for an MFC-based BOD_Q sensor.[13] Currently, two major limitations hinder the practical application of MFC-based BOD_Q sensors: (a) the measured BOD_Q values are systematically underestimated, and (b) the detection process remains time-consuming.[14-16] The main reason is that the output current depends strongly on external resistance, which is closely related to electron transfer efficiency.[17] Furthermore, the extended current-time curves are attributed to microbial endogenous consumption rather than BOD_Q contributions.[16] Therefore, optimizing both the external resistance and the detection time is essential to improve the performance of MFC-based BOD sensors.

In this study, the effects of varying external resistances and current-time curves on the accuracy and detection time of

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BODQ measurements are systematically analyzed using an MFC sensor. By comparing four resistances (1000 Ω, 500 Ω, 100 Ω, and 10 Ω) and analyzing different points along the current–time curve, this work aims to identify the optimal conditions for obtaining high accuracy and low detection time. Based on this, actual measurements of BOD_Q in sewage were carried out. The findings not only provide insights into the design of more practical MFC-based BOD_Q sensors but also contribute to the broader goal of developing rapid and reliable water quality monitoring tools for real-world wastewater management.

Methods

MFC-based BOD_Q sensor construction:

A typical single-chamber (polymethyl methacrylate) MFC was constructed as the BOD_Q sensor, with solution volumes of ~6 mL. The anode consisted of a cylinder-shaped carbon felt (Sanye Carbon Co. Ltd., Beijing, China) of 3 cm in diameter and a thickness of 0.3 cm. Before use, the carbon felt was cleaned with a 5% acetone solution to remove surface impurities. The air cathode was made of cylinder-shaped carbon cloth with a catalyst (Pt/C, 0.5 mg/cm^2) of 3 cm in diameter and a thickness of 0.1 cm. A titanium ring was used as a current collector. The cathode and anode were connected by an external resistor (1000 Ω, 500 Ω, 100 Ω, 10 Ω). These materials were kindly provided by the School of Environment, Tsinghua University, China.

Startup of the MFC-based BOD_Q sensor:

The inoculum was sourced from the effluent of an MFC reactor that had been operated for a long time in the laboratory at Tsinghua University. During startup, the anolyte was made of artificial wastewater, which contained 1.64 g/L sodium acetate (NaAc), 0.1 g/L $MgCl_2{\cdot}6H_2O$, 0.31 g/L NH_4Cl, 0.1 g/L $CaCl_2{\cdot}2H_2O$, 3.4 g/L $K_2PO_4{\cdot}3H_2O$, 4.4 g/L KH_2PO_4, 5 mL/L vitamins solution, and 12.5 mL/L trace minerals solution.[17] During testing, real wastewater was further used to verify the performance of the MFC sensor. Real wastewater was sourced from a municipal WWTP in China.

When artificial wastewater or real wastewater was supplied to the anode chamber of MFC, the anode biofilm oxidized the organic matter contained in wastewater and released electrons e- and protons H^+. Electrons e- were then transferred as current through the resistance, while O_2 in the atmosphere is reduced into H_2O by gaining electrons e- and H^+ in the Pt/C cathode.

Data acquisition and BOD_Q calculation:

The current was observed by measuring the voltage across the anode and the cathode with the multimeter.

Voltage data were recorded every second, and the operation period was approximately 8 hours. The corresponding current was calculated using Ohm's law (Equation 1).

$$I = \frac{U}{R} \qquad \text{[Equation 1]}$$

Where I is the current (A), U is the voltage measured (V), and R is the external resistance (Ω).

Following previously established methods,[4] the coulomb quantity (Q-C) was calculated by integrating the current (I-mA) over time (t-h) (Equation 2):

$$Q = \int_0^t I(t)dt \qquad \text{[Equation 2]}$$

According to the coulomb quantity, the BOD_Q could be calculated using Equation 3:

$$BOD_Q = \frac{8000}{FV} \times Q\left(\text{mg } O_2 \cdot L^{-1}\right) \qquad \text{[Equation 3]}$$

Where F is the Faraday constant (96485 C/mol), and V is the effective volume of anolyte (L).

The Cyclic voltammetry (CV) test was performed by an electrochemical workstation, with a scan rate of 10 mV/s at a potential window from –0.7 V to 0.4 V *vs.* Ag/AgCl. All experiments were operated at room temperature, and each experiment was conducted twice. The Origin 2024 software performed data treatment.

Results and Discussion

This study constructed a single-chamber air-cathode MFC as the sensor for BOD detection (Figure 1a). Electroactive biofilm growing on the anode oxidizes organic matter to generate electrons and protons (Figure 1b). The electrons were transferred to the cathode via an external circuit, while the protons migrated to the cathode driven by the electric field. At the cathode, with the aid of catalysts, water was formed through the oxygen reduction reaction. By monitoring the external current using electrochemical equipment, the corresponding coulomb quantity could be calculated, which was further correlated to determine the BOD_Q value (Figure 1b).

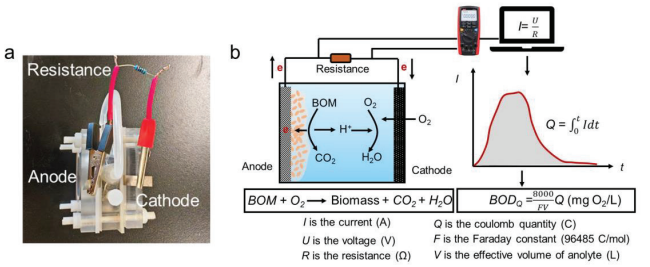


Figure 1: Schematic of MFC sensor-based BOD_Q detection. (a) photograph of the MFC sensor, (b) the test method of BOD_Q. This figure illustrates the device for the MFC sensor and the basic principle of BOD_Q detection.

MFC sensor start-up:

The inoculum was sourced from the effluent of an MFC reactor that had been operated for a long time in the laboratory at Tsinghua University. After centrifugation (5000 rpm), the inoculum was added to artificial wastewater to start the MFC sensor. Each cycle (~20 hours) represents the complete process of the voltage rising and then falling after the addition of the nutrient solution. The max output voltage stabilized at 0.4 V after two cycles (Figure 2a), and the fact that the maximum voltage did not increase in the next cycle indicated a successful startup. The Cyclic voltammetry (CV) curve revealed a distinct redox peak, confirming the successful startup of the

MFC sensor (Figure 2b). After that, the MFC sensor would be used for BODQ detection, and some effect factors (e.g., resistance, detection time) would also be further analyzed to obtain a high performance of the MFC-based BOD_Q sensor.

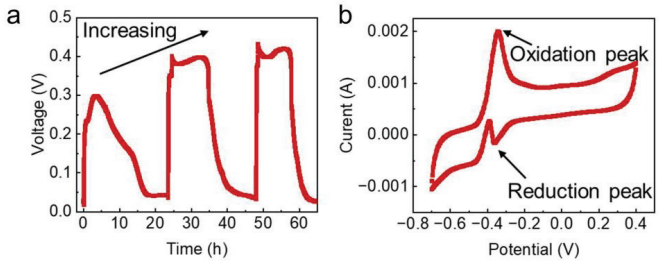
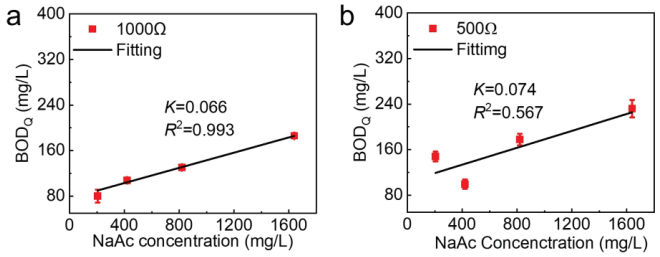


Figure 2: MFC sensor start-up. (a) the output voltage, (b) cyclic voltammetry curves. This figure indicates the successful startup of the MFC sensor.

Effects of external resistances on MFC-based BOD_Q sensor:

Resistance is a key factor in developing the high performance of MFC-based BOD_Q sensors. Therefore, the effects of different resistances (10 Ω, 100 Ω, 500 Ω, 1000 Ω) on BOD_Q were first analyzed in this study. Results indicated that the MFC sensor with 100 Ω had both the highest sensitivity (K=0.197) and linearity (R^2 =0.993) (Figure 3c). It was meant that 100 Ω represented the optimal resistor condition for an MFC sensor in assessing the biodegradability of wastewater. Although 1000 Ω and 10 Ω also showed high linearity, the sensitivity was too low to reflect the biodegradability of wastewater (Figure 3a, d). The 500 Ω performed poorly, with a low sensitivity and linearity, suggesting an underestimated indicator (Figure 3b). In conclusion, 100 Ω was an optimal resistor for the MFC-based BODQ sensor.

This study demonstrated that external resistance played a crucial role in the MFC-based BOD_Q sensor.[17] When resistance was set too low (10 Ω), the circuit demanded more current than the microbial community could sustain, leading to a rapid decline in voltage and unstable output (Figure 3d). This suggested that microorganisms were unable to maintain efficient electron transfer under excessive load. On the other hand, when resistance was set too high (1000 Ω), electron flow was restricted, suppressing microbial metabolism and lowering overall power generation (Figure 3a). The performance at 100 Ω suggested that the external resistance closely matched the system's internal resistance, allowing efficient electron transfer and maximizing both sensitivity and linearity (Figure 3c). Therefore, 100 Ω offers the best balance between sensitivity and linearity, making it more suitable for BOD_Q detection.



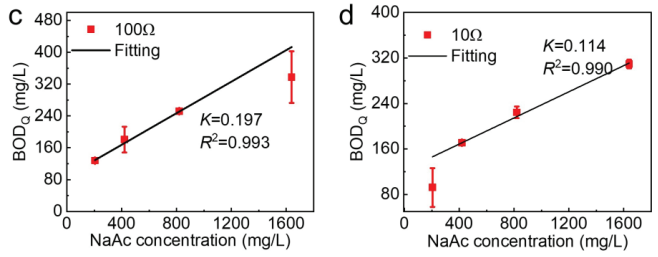


Figure 3: Effect of different resistances on MFC-based BOD_Q sensor. (a) 1000 Ω, (b) 500 Ω, (c) 100 Ω, (d) 10 Ω. The *K* value represents the slope of dose (NaAc concentration)-effect (BOD_Q) curves, and R^2 indicates the fitness of the data. The results indicated that an external resistance of 100 Ω had the highest sensitivity (*K*=0.197) and a relatively high linearity (R^2=0.993), matching the system's internal resistance to maximize electron transfer.

Effects of detection time on MFC-based BOD_Q sensor:

Detection time is also a key factor in developing the high performance of MFC-based BOD_Q sensors. This study selected three specific points in the *I-t* curves, where I_{max} was the point of maximum current, I_d was the point where the current declined at the fastest rate, and I_0 was the point of lowest current (Figure 4a). Figure 4b illustrates the relationship between NaAc concentration and BOD_Q at three specific detection points. The results demonstrated that BOD_Q values derived from I_d exhibited superior sensitivity and linearity, enabling accurate assessment of wastewater biodegradability (Figure 4b). In contrast, I_0, while maintaining high linearity, showed the lowest sensitivity, indicative of an unstable BOD_Q response. I_{MAX} presented a balanced performance in terms of sensitivity and linearity; however, its overall values were consistently lower than those obtained from I_d. Figure 4c compares the required detection time for each specific point. The ranking order of detection time was as follows: I_{max} < I_d < I_0 (Figure 4c). Although I_{max} had the shortest detection time, it was difficult to reflect wastewater biodegradability accurately. Based on careful consideration, ID was identified as the most suitable detection time point, with the monitoring duration on the order of several hours.

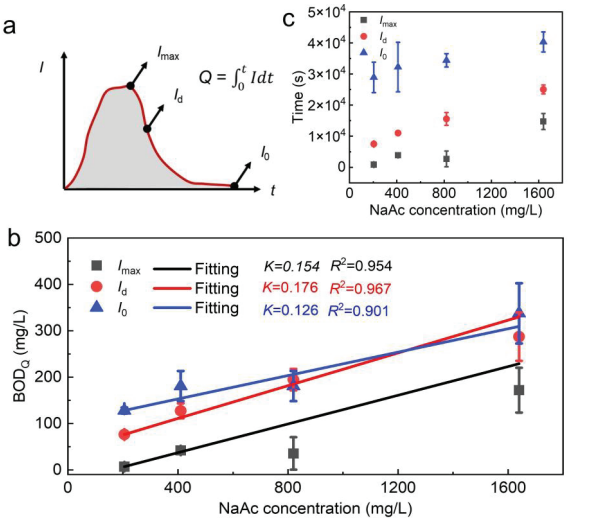


Figure 4: Three signal analysis methods (I_{max}-peak current, I_d-fastest decay, I0- lowest current). (a) the calculation principle, (b) BOD_Q values, and (c) the test times. The results indicated that the fastest decay point (I_d) had the highest sensitivity and linearity value while maintaining an applicable detection time, reducing detection time to several hours while maintaining data consistency.

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The selection of detection time is closely related to microbial metabolism and the degradation of organic matter. The maximum current corresponds to the peak rate of microbial organic matter degradation. However, this did not signify that the microorganisms had completely consumed the biodegradable organic matter in the wastewater; at this point, the calculated BOD_Q value was significantly underestimated. When the current decreased to approach zero, it indicated that the microorganisms had not only consumed the available biodegradable organic matter but had also depleted their endogenous reserves. Consequently, allowing the current to approach zero requires a very long time, which is impractical for a sensor that requires a relatively short detection time. The point at which the current decreased most rapidly emerges as the optimal termination time for BOD_Q detection, as it corresponds to the rapid degradation of organic matter in wastewater by microorganisms. While I_d serves as a reliable proxy, its exact timing depends on the specific substrate composition of wastewater and the microbial community structure within the anode biofilm. Despite these biological variables, utilizing I_d provides the most effective balance of sensitivity, linearity, and detection time required for practical applications.

Validation in real wastewater measurement:

The two optimal conditions were further applied to real wastewater samples. Figure 5a presents the BOD_Q test results of samples collected from a municipal WWTP (Wastewater treatment plant of Huai'an Qingjian, Jiangsu province, China). Each sample was set as 1#, 2#, and 3#, corresponding to raw wastewater, one-fold dilution, and two-fold dilution, respectively. To ensure the accuracy of the tests, all tests were conducted at room temperature, the pH was adjusted to neutral, and the dissolved oxygen was controlled below 1 mg/L. Figure 5b showed the COD test results, and Figure 5c showed the biodegradability evaluation of wastewater samples. The results showed that as the dilution factor increased, the BOD_Q concentration gradually decreased (Figure 5a). The COD concentration showed a similar trend (Figure 5b). The biodegradability assessment, based on the BOD_Q/COD ratio (Figure 5c), revealed that the ratio for the raw wastewater sample (1#) was greater than 0.3, representing good biodegradability. In contrast, the diluted samples 3# and 4# had ratios below 0.3, representing poor biodegradability.

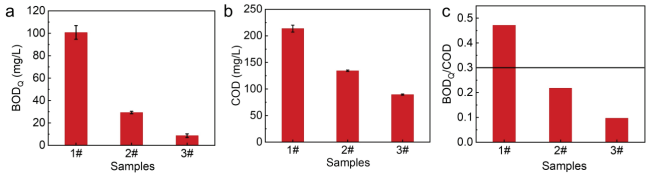

Figure 5: BOD_Q test and biodegradability evaluation of real wastewater using MFC-based sensors. (a) BOD_Q test results, (b) COD test results, (c) biodegradability evaluation. The results demonstrated that BOD_Q could be used for evaluating the biodegradability of real wastewater.

Real wastewater measurement suggested that wastewater's biodegradability was poorer under low COD conditions, possibly because the proportion of non-biodegradable organic matter was slightly higher, which decreases their activity. Electroactive microorganisms generated fewer electrons under low COD conditions since the concentration of complex, non-biodegradable molecules is not affected by it.

Limitations and future perspectives:

While the proposed MFC-based BOD_Q sensor demonstrates significant potential for rapid and accurate wastewater assessment, limitations must be acknowledged. The current study primarily evaluated the sensor under controlled laboratory conditions; real-world wastewater often contains complex matrices, including heavy metals and extreme pH fluctuations, which could inhibit electroactive biofilm and affect long-term sensor stability. Therefore, future research should focus on testing the sensor's robustness across a wider variety of wastewaters.

■ Conclusion

The investigation showed the potential of MFC sensors as a rapid and reliable method for measuring BOD_Q values in wastewaters. By testing different external resistances and observation time points, it was defined that 100 Ω is the most effective resistance for achieving both sensitivity and linearity. Furthermore, the analysis of time-based current data showed that using the point where the current declines at the fastest rate as the observation point provides overall the most accurate performance for sensitivity, linearity, and detection time. The combination of these two optimizations was verified in a real wastewater measurement. It significantly enhances the practicability of MFC sensors, reducing detection time from days to hours while maintaining data integrity. This finding represents a critical step toward the development of real-time water quality monitoring tools essential for environmental management.

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