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Design, optimization and application of a highly sensitive microbial electrolytic cell-based BOD biosensor

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ABSTRACT

Biochemical oxygen demand (BOD) is an important biochemical indicator for determining the degree of water pollution and guiding the design of wastewater treatment processes. BOD sensors based on microbial electrochemical technology can conduct real-time online monitoring of organic matter and have attracted extensive attention. However, research on microbial electrolytic cell (MEC)-type BOD sensors is at the stage of theoretical exploration. Here, we designed and optimized a highly sensitive MEC-type BOD sensor by screening inoculants, comparing electrode materials, and optimizing the reactor configuration. The results showed that effective means to optimize a BOD sensor for fast activation and sensitive testing included the inoculation of the MEC reactor effluent with large amounts of biomass and highly active bacteria, selection of carbon felt electrodes with excellent adsorption and permeability, miniaturization of the reactor, regulation of suitable electrode spacing, and design of the penetrating fluid structure. Then, the optimized sensing system was applied to determine the BOD concentration in model solutions of sodium acetate in a laboratory environment, where it accurately measured BOD concentrations in the range of 10–500 mg/L and maintained good parallelism during long-term operation. Next, the MEC-type BOD sensors were put into practice in the field as an alarm for accidents at an actual sewage plant. The whole BOD sensing system was quickly assembled on site and started up, and it gave an early warning shortly after the concentration of organic matter in the water suddenly increased, thus showing a high potential for engineering applications. This study broadened the domains of application of MEC-type BOD sensors in environmental monitoring, and promoted the development of technological innovation in water ecology and environmental monitoring.

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Efforts to reach carbon neutrality are increasing the demands on water quality monitoring and water pollution control. The concentration of organic matter is the main parameter of the quality control process in each step of the wastewater treatment process and an important gauge for evaluating the effectiveness of each stage (Liu et al., 2016). However, biochemical oxygen demand (BOD) is more instructive for evaluating the biochemical treatment process. The present BOD monitoring methods (Jouanneau et al., 2014) include standardized, modified reference, photometric and manometrical methods, but they cannot realize real-time monitoring of BOD, let alone meet the regulatory requirements for in situ real-time online monitoring of organic carbon sources during actual wastewater treatment processes. In recent decades, bioelectrochemical systems (BESs) have emerged as new and attractive online biosensors, showing rapid, broad-spectrum and

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Table 1
Parameter design for different configurations of reactors.

Reactor configuration	MEC-1	MEC-2	MEC-3	MEC-4
Volume (mL)	56	28	20	20
Electrode spacing (cm)	2	1	6	3
Flow Mode	Side stream	Side stream	Penetrating stream	Penetrating stream

efficient response to organic pollutants in water; microbial fuel cell (MFC) and microbial electrolytic cell (MEC) BOD sensors are the most promising applications. Compared with an MFC sensor, an MEC sensor operates under a constant voltage power supply, and the system itself has a certain anti-interference ability. The detection data have better parallelism when they are obtained from the same equipment configuration (Adekunle et al., 2019; Kadier et al., 2016; Saravanan et al., 2020). However, the current research on MECs mostly focuses on resource recycling, and its application in the field of BOD sensing is still at the stage of theoretical exploration (Hua et al., 2019).

In microbial electrochemical sensing systems, an electroactive biofilm (EAB) is the primary source of the electrical signal output and determines the signal response process and sensitivity (Erable et al., 2010; Qi et al., 2021). The key to high-performance EABs is exoelectrogens (Ren et al., 2021; Zhao et al., 2021), which necessitates screening to identify inoculants that can efficiently and consistently deliver electroactive bacteria (Tang et al., 2017). Wastewater, aerobic sludge, seawater, marine sediment and, to a lesser extent, soil are the most common sources of inoculum for culturing EABs. Moreover, the selection of the working electrode material and its placement in the reactor directly affect the electron transfer efficiency of the BES, thus affecting the sensitivity of the sensor response (Breheny et al., 2019; Cai et al., 2020; Mier et al., 2021). The electrode material used as the support skeleton of the EAB should have suitable functional parameters, such as bioaffinity, water permeability, adsorption, catalytic ability and electron-collecting capacity (Fan et al., 2021; Hindatu et al., 2017; Sonawane et al., 2017). Additionally, the reactor configuration is a key factor in sensor performance, which directly affects the detection limit, baseline current and sensitivity of the sensor by varying the volume and hydrodynamics of the internal fluid (Cecconet et al., 2018; Fujii et al., 2021; Xiao et al., 2021). Using hydrodynamic simulation and computation to explore the effect of the flow pattern inside the reactor on the mass transfer process and fluid dead zone volume, researchers found that when fluid flows laterally through the work electrode, it tends to form a boundary layer on the biofilm that decreases the mass transfer velocity and sensitivity of the EAB (Yi et al., 2020a). Conversely, the sensitivity and resolution of the sensor are greatly improved by penetrating flows and rational design of the inlet/outlet locations, apertures and flow rates.

Based on the theory of anodic electroactive biofilms, this study proposed a complete design, optimization and application scheme for MEC-type BOD sensors. The performance of the sensor was enhanced by optimizing the inoculant, electrode material and reactor configuration. Together with the supporting data acquisition and wireless transmission equipment, a BOD monitoring system was made and validated in simulated BOD testing with solutions of NaAc. Immediately afterward, the entire BOD monitoring system was put into practice in the field as a warning system at an actual wastewater treatment plant, and further engineering and commercialization research solutions were proposed to provide a theoretical basis and technical support for the development of fast and efficient sensitive BOD real-time online monitoring products.

1. Materials and methods

1.1. Construction of the sensor

Four types of single-chamber MEC reactors (Fig. S1) were designed and used to investigate the effect of different reactor configurations and electrode materials on the performance of BOD sensors. All the reactor bodies were made of acrylic cubes with internal cylindrical cavities where a constant potential of 0.7 V was steadily applied on a two-electrode system through a power supply module in the wireless data acquisition equipment that was developed in this laboratory. The main differences between the four types of reactors were their internal volumes, the spacing of the two electrodes and the mode of fluid flow (Table 1). For the optimization of the electrode materials, three different materials, 100 mesh 304 stainless steel mesh (SSM), 2 mm carbon felt (CF) and carbon cloth (CC), were selected and pretreated by soaking in acetone for 12 h, repeatedly rinsing with ethanol and distilled water, and gradient heating (5 °C/min) to 450 °C for 30 min of continuous calcination before use (Wang et al., 2009).

1.2. Activation, operation and maintenance of the BOD sensors

River sediment leachate, MFC anode effluent and stacked MEC effluent were selected as inocula for sensor activation; the dual-chamber MFC (Fig. S2A) and stacked MEC (Fig. S2B) had been used in our laboratory for more than two years. During the start-up phase, the BOD sensors were fed in sequential batch mode, and the purified electrolyte (Lovley and Phillips, 1988) containing 50 mM phosphate buffer solution (PBS, Na_2HPO_4, 4.58 g/L; NaH_2PO_4, 2.13 g/L; NH_4Cl, 0.31 g/L; and KCl, 0.13 g/L), 12.5 mL/L trace minerals, 5 mL/L vitamin solution and 1 g/L sodium acetate medium was replaced when the current was less than 0.1 mA (approximately every 2 days). The dissolved oxygen in the system was removed by continuous aeration with N_2/CO_2 (4:1) for 30 min before the electrolyte was refreshed, and this process was recorded as a cycle. When three cycles of repeated maximum current output were detected, the activation was considered complete, and the sensors were then converted to continuous flow mode operation to maintain the activity of the EAB and stable baseline current signal. Upon completion of the BOD test, the fresh electrolyte was refreshed via a continuous stream for sensor operation and maintenance. All experiments were conducted in triplicate at room temperature (25 ± 1 °C).

1.3. The testing and calculations of the BOD sensors

The performance of the BOD sensors was tested and analyzed by multifunctional electrical signal acquisition equipment integrating a power supply module, data acquisition module and data wireless transmission module, which simplified the structure of the sensing system and performed real-time acquisition and wireless transmission of current signals (Fig. S3A). Additionally, the equipment realized the simultaneous operation and data acquisition of ten MEC-type BOD sensors, whose data acquisition interval was adjusted autonomously within the range of 50 ms–10,000 s, and the current range was 0.1–6 mA. The complete sensing system (Fig. S3B) tested the BOD of simulated wastewater in continuous flow mode, where 5 mmol/L PBS was used to simulate the low buffered state of the actual wastewater, while different concentrations of NaAc were added to convert various concentrations of BOD (1 mg NaAc = 0.52 mg BOD_5). Moreover, a small self-priming pump (rated power 5 W) and a flow valve to control the influent rate of 10 mL/min were used to simulate a continuous stream of wastewater.

Start-up time (t_s), response time (t_r), baseline current (Ia), current variation (ΔI), and sensitivity (S) are all common metrics used to describe the performance of BOD sensing systems. Here, t_s was defined as the time required to reach the peak current during the start-up phase, while t_r was the time taken for the current to respond to BOD changes during the test phase. ΔI was the current variable after a change in

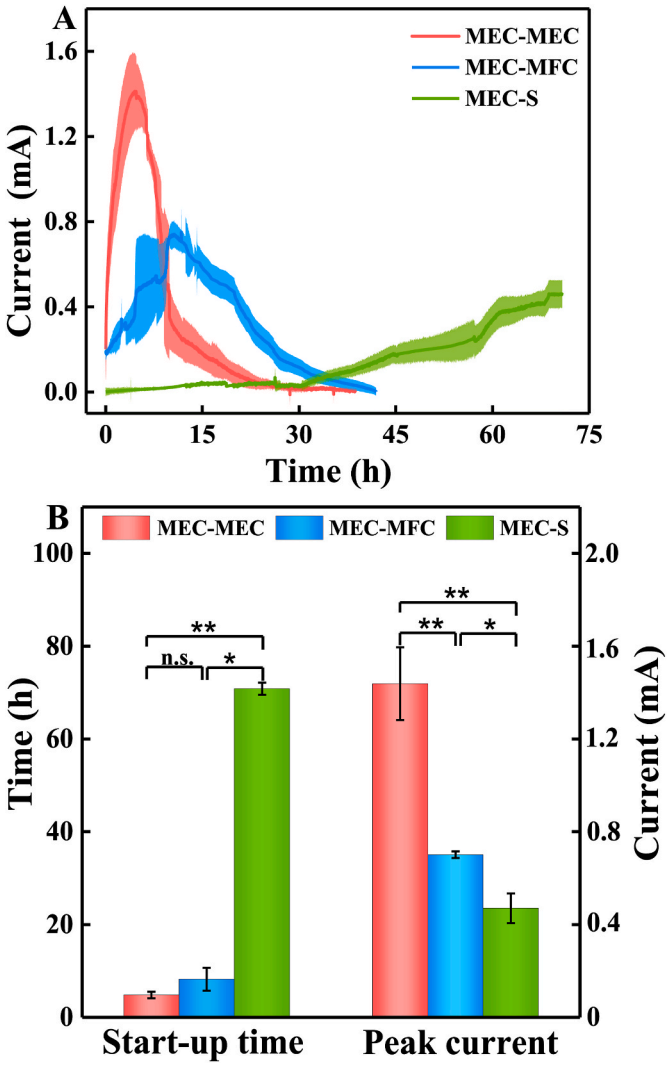

Fig. 1. Time-current curves (A) and comparison of electrochemical performance (B) of MEC-type sensors domesticated with different inoculants.

organic concentration and was calculated as $\Delta I = Ib - Ia$, where *Ib* was the first 5-min-stable current after a certain change in organic concentration and *Ia* was the stable baseline current before the test, which is caused by instrument noise. S was obtained by fitting ΔI to the BOD concentration (c) through the linear equation $\Delta I = kc + b$, where a higher value of *k* indicated a more sensitive response of the system. In field tests, BOD was estimated from the real-time current data using above linear equation.

1.4. Test applications in actual wastewater plants

The actual application of the BOD sensor was conducted at a wastewater treatment plant used mainly to treat industrial wastewater from pig farming and wastewater from employees within the plant. The sewage flowed through a grid that removed large-size suspended solids and then entered and circulated in an aerobic/anaerobic tank, where the aerobic pond was equipped with an aeration pump at the bottom to increase the amount of dissolved oxygen and provide air flotation, while the anaerobic pond had a design similar to that of the clarifier, and a large amount of activated sludge filler was added for adsorption and biochemical treatment to remove organic pollutants. The treated wastewater flowed into a pond used for fish breeding through further flocculation, sedimentation, disinfection and deodorization.

The start-up of the BOD sensor for the field test was carried out in two

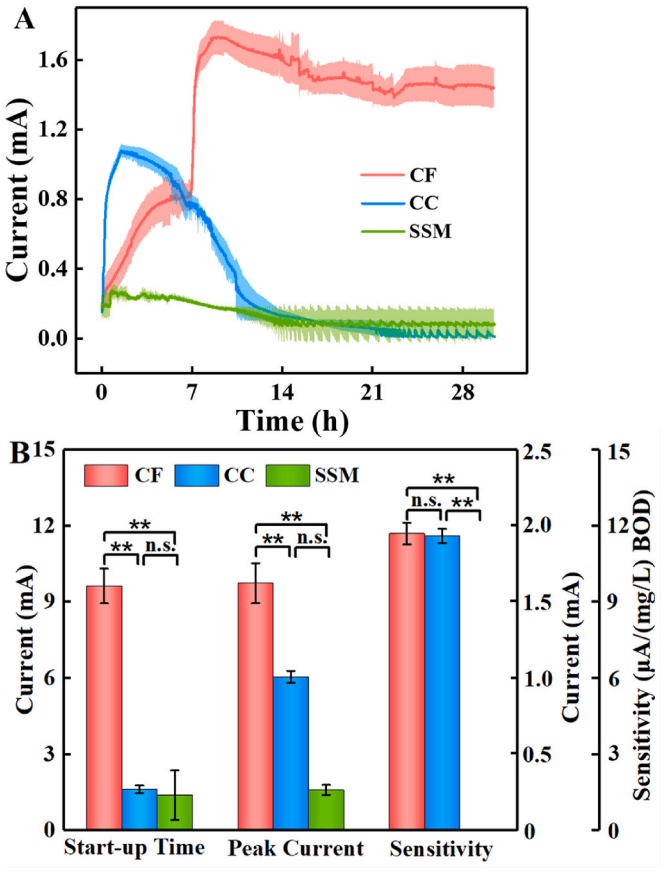

Fig. 2. Time-current curves (A) and comparison of electrochemical performance (B) of MEC-type sensors with different electrode materials.

ways: one MEC sensor contained an EAB that had undergone pre-start-up in our laboratory (Pre-start-up), and another MEC sensor contained an EAB that had been started up with indigenous bacteria from the on-site wastewater plant (On-site). Both sensors were used for early warning of BOD changes in the anaerobic tank, and the flow rate was set to twice that of the laboratory test (20 mL/min) considering the large amount of suspended material in the effluent. Unfortunately, the site conditions prevented the installation of an LAN over long distances, so a short-range data collection solution was adopted, which led to the installation of the data receiving and checking device outdoors as well (Fig. S4). Additionally, the chemical oxygen demand (COD) content was measured using COD test kits (KYORITSU, Japan) for auxiliary verification during the BOD warning process.

2. Results and discussion

2.1. Effect of different inoculum sources on the activation and operation of BOD sensors

The inoculant was the most influential parameter affecting the performance of the BOD sensor, as highly active EABs were more sensitive than less active EABs to environmental factors. The effect of different inocula on the sensor was fully reflected in the initial activation stage (Fig. 1). The sensor initiated with river sediment leachate (MEC-S) as the inoculum had the longest start-up time of 71 ± 1.3 h, which was 8.7 times longer than that of the sensor inoculated with dual-chamber MFC effluent (MEC-MFC, t_s = 8.2 ± 2.5 h). In contrast, the sensor incubated with stacked MEC effluent (MEC-MEC) required only 4.8 ± 0.7 h to reach an initial peak current as high as 1.4 ± 0.16 mA, which was 198% and 100% higher than that of MEC-S (0.47 ± 0.06 mA) and MEC-MFC (0.7 ± 0.01 mA), respectively, fully indicating that the sensing

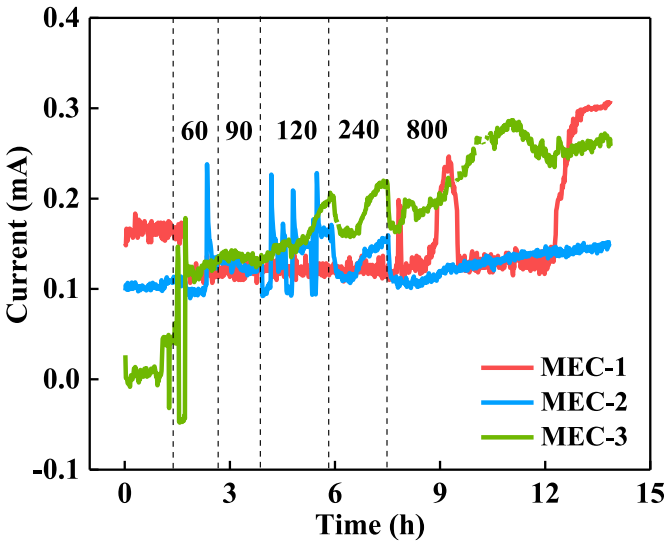

Fig. 3. Response currents of larger volume sensors (MEC-1) to sodium acetate.

element of the MEC-MEC had higher electrochemical activity than the others. With subsequent cycles of acclimation, the peak currents of the BOD sensors from different inocula increased compared to the initial cycle, but the trends in the differences between them did not change. Combined with the results of our previous studies on the microbial community of similar systems (Du et al., 2019), the following reasons were analyzed: Microorganisms in river sediments were not screened by electric fields, and the biomass of exoelectrogens was low and not dominant in abundance, which was detrimental to the rapid activation of the sensors. Compared to the MFC effluent, the exoelectrogens in the MEC effluent screened with a strong electric field had a higher electrical activity, which drove the BOD sensor to start up more quickly and provide more efficient electrochemical performance.

2.2. Effect of different electrode materials on the performance of BOD sensors

The electrode material was an important factor affecting the BOD sensors. In this study, three anode materials, SSM, CF and CC, were used to construct BOD sensors in the same reactor configuration (MEC-4). Comparison of the three electrode materials for MEC electrochemical performance and sensitivity to low organic concentrations (BOD <50 mg/L) showed that the SSM anode still had a low current (0.27 ± 0.2 mA) after multiple cycles of incubation and a large signal-to-noise ratio (SNR), which was prone to large errors (Fig. 2). This was because the bioaffinity and adsorption on the SSM were mediocre, and it was difficult for a stable EAB to form on its surface. The EAB had poor resistance to environmental shocks, such as high continuous flow rates, that prevented it from maintaining a stable baseline current, resulting in no response to low concentrations of BOD addition and a sensitivity of almost 0. The sensors with the CF and CC as electrode materials showed excellent starting and response behavior. Interestingly, compared with the shorter start-up time of the CC anode (1.6 ± 0.15 h), the CF anode required 9.6 ± 0.69 h to reach a maximum current of 1.6 ± 0.13 mA because the CF had a larger specific surface area and required more biomass than the CC to reach maximum power production; this also explained why the peak current was 60% higher with the CF anode than with the CC anode (1.0 ± 0.04 mA). Furthermore, the response sensitivities of the CC and CF anodes to BOD were not significantly different (CF, 11.7 ± 0.42 μA/(mg/L); CC, 11.6 ± 0.28 μA/(mg/L)), but the structure of the CC was looser and more inconvenient to handle and use, so the CF was the best choice in this study.

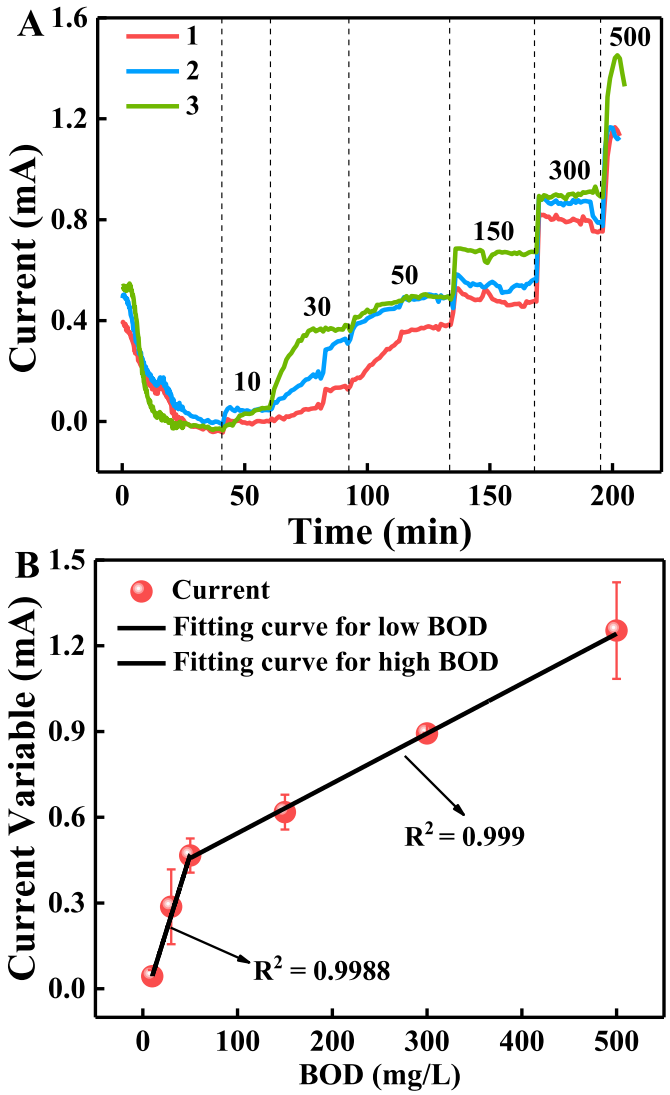

Fig. 4. Response curves of the sensors to BOD changes simulated by acetic acid (A) and segmented fit curves of BOD versus current signals (B). The numbers were the BOD concentration (mg/L) accounted for from sodium acetate, 1,2,3 indicated the sensors in triplicate.

2.3. Effect of different reactor configurations on the performance of BOD sensors

The reactor configuration was another important factor affecting sensor performance. The larger volume sensor (MEC-1) responded significantly only to BOD concentrations greater than 200 mg/L, with a high detection limit and response time longer than 1.5 h (Fig. 3), which could not meet the demand for high sensitivity real-time monitoring of BOD. The electrode spacing could modulate the electrochemical performance of the sensor by affecting the electric field strength and decreasing the electrode spacing was conducive to the sensitivity of the sensing elements. Compared with the side-stream mode, the vertical penetration stream mode more effectively refreshed the internal electrolyte with continuous flow, thus providing a more accurate response to shock caused by organic contaminants (Jiang et al., 2017).

2.4. Response of the BOD sensor to sodium acetate

The BOD response test of the optimized MEC sensors was carried out by varying the sodium acetate concentration at different times during continuous flow of the influent. The variation in the electrical signal

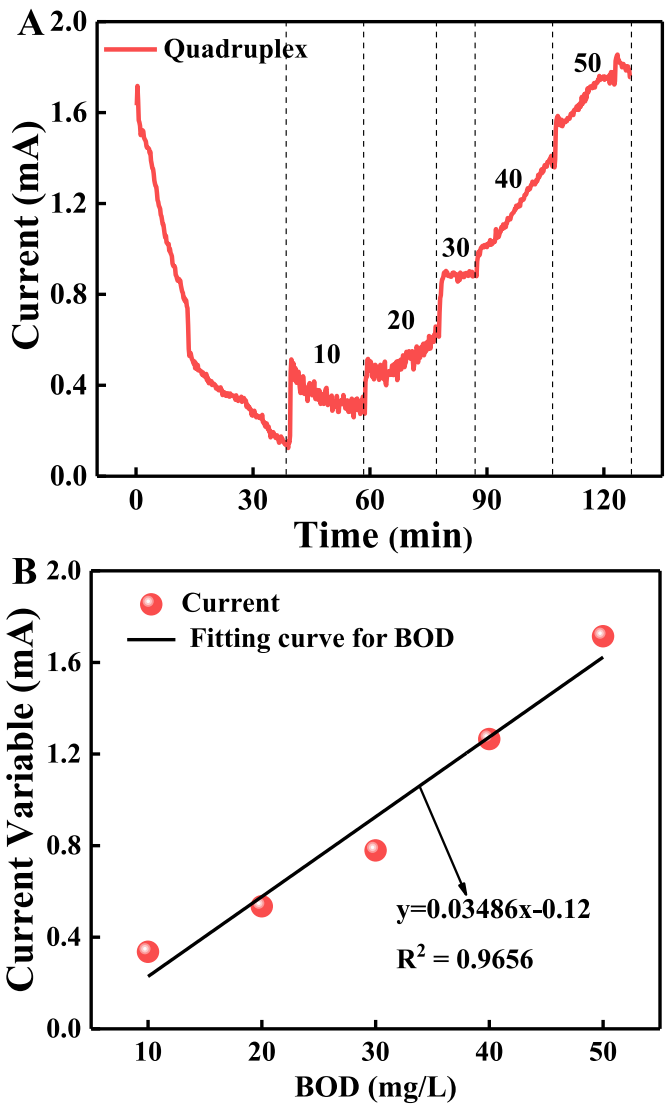

Fig. 5. Current response curve (A) and fitted curve of BOD vs. current signal (B) for four sensors in parallel to monitor low concentration of BOD. The numbers were the BOD concentration (mg/L) accounted for from sodium acetate.

demonstrated that the current parallelism for the response of the BOD sensors in triplicate was high and within the detection range of 10–500 mg/L (Fig. 4A). The calculated sensitivity of the sensor showed two linear ranges (10–50 mg/L, 50–500 mg/L) between the current variables and the BOD concentration, with fits as good as 0.9988 and 0.999, respectively (Fig. 4B), indicating that the sensor was very accurate in determining BOD in the range of 10–500 mg/L, which laid a solid foundation for its application in real wastewater plants.

The MEC sensors required a certain concentration of BOD to maintain the electrical activity of the exoelectrogens. When the theoretical BOD concentration of the test water sample was less than 30 mg/L, the current signal output from a single sensor was small (<0.4 mA), which could easily cause large errors. To solve this problem, four MEC sensors were connected in parallel to accurately test water with lower BOD concentrations (10–50 mg/L) by increasing the current signal. As expected, the cumulative amplification of current was achieved by connecting four sensors in parallel, which solved the problems of large errors and poor repeatability of sensor measurements at low BOD concentrations and achieved highly accurate determinations of BOD concentration changes (Fig. 5). This also provided insight into the design of sensors for detecting low BOD concentrations in water sources by integrating a number of sensors to achieve high-throughput monitoring.

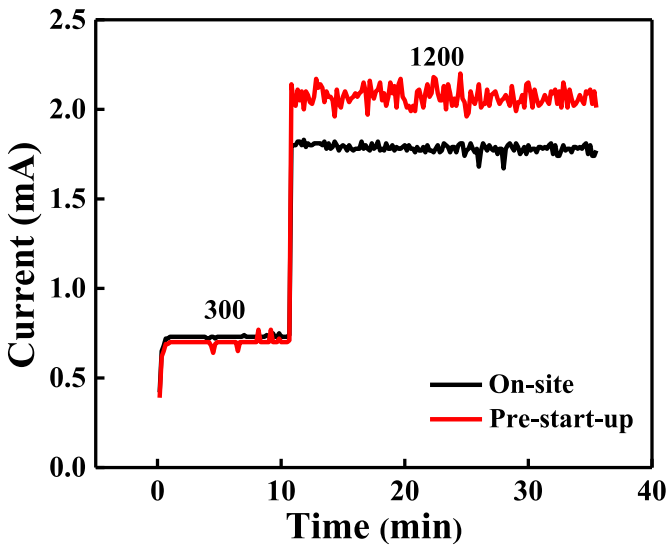

Fig. 6. Current response curve of the MEC sensor during the incident, the figure is the crude COD concentration.

2.5. *Effectiveness of BOD sensors in actual wastewater plants*

The BOD sensor was used to monitor the anaerobic pond on site, and the dissolved oxygen level in the area was kept below 0.1 mg/L for a long time as measured by the dissolved oxygen meter, meeting the conditions for the use of the MEC sensor. In the start-up phase, a stable baseline current developed within 12 h for both sensors (On-site and Pre-start-up), and the sensor that was prepared with the pre-start-up method had a faster start-up time due to its more mature EAB. During the operation phase, both sensors maintained a relatively stable baseline current (0.7 mA), and each had excellent parallelism under the normal operation of each step in the wastewater treatment process. During the monitoring phase, when the activated sludge filler used for adsorption and biochemical reactions was washed out due to equipment control errors, the BOD concentration of the water in the detection section rose rapidly, and the current signal of the MEC sensors increased rapidly from the baseline value of 0.70 mA–1.7 mA (On-site) and 2.0 mA (Pre-start-up) in a very short time (<1 min), indicating that the sensors could achieve rapid warning of BOD changes (Fig. 6). Furthermore, the COD concentration in the tested wastewater at the time of an accident was detected to be approximately 1200 mg/L, which was four times higher than that in the absence of an accident (approximately 300 mg/L), reflecting to a certain extent that the BOD sensor had the abilities of fast start-up, long-term stable operation, and rapid response to provide an early warning of concentration changes in organic materials during actual wastewater applications. Compared with other electrochemical BOD biosensors (Table 2), we developed a MEC-type BOD sensor with both large detection range, quick start-up, real-time response, low cast, and ability to detect real wastewater. However, the actual BOD concentration in the wastewater before and after the accident could not be determined due to the environmental conditions in the field, so it is necessary to explore the transformation model of the current signal and BOD concentration in practical applications and conduct further field tests.

Alternatively, further modifications and enhancements of MEC-type BOD sensors are needed to realize commercial applications. For complex scenarios with large fluctuations in biochemical indicators, membrane modules with strong adaptability, stable community composition and function should be constructed to achieve long-term stable operation. The design of integrated packaging of commercial products and devices

Table 2
Some typical MxC-based biosensors for BOD detecting.

Type of MxC	Inoculum Source	Electrodes		Detection range	Response Time	Start-up Time	Detect real wastewater	Reference
		Anode	Cathode					
Double-chambered MFC	long-term stable large-scale MFC	carbon cloth	carbon cloth	37.5–375 mg/L	1–18 h	3–5 d	Yes	Gao et al. (2020)
Power assisted MFC	wastewater	recycled granular graphite	stainless-steel mesh	<316 mg COD/L	10–60 min	168 d	No	Fernandez-Gatell et al. (2022)
Single-chamber MFC	continuous flow MFC	carbon cloth	carbon cloth	40–164 mg/L	2.8–12.0 min	3–5 d	No	Di Lorenzo et al. (2014)
Three-electrode MEC	*Shewanella loihica*	carbon cloth	platinum plate	5–435 mg/L	60–300 min	2–3 d	No	Yi et al. (2020b)
Two-electrode MEC	stacked MEC effluent	carbon cloth	stainless-steel mesh	10–500 mg/L	<5 min	1.6 h	Yes	This study

for data collection and entry into cloud storage are also required before installing and commissioning equipment in the field and testing automated methods of data collection. According to the results of this study, the traditional linear simulation technique was critically limited. The real-time current variation in the sensor contained numerous pieces of information characterizing the BOD concentration, and simply extracting the steady-state current for fitting caused the loss of a large amount of valid information. Therefore, the development of accurate signal-indicator models using artificial intelligence is of paramount importance.

3. Conclusions

The inoculant, electrode material, and sensor configuration are key factors that enhance sensor performance. The start-up time and power production performance of the sensor were improved when the sensor was initiated with stacked MEC effluent as the inoculum. Carbon felt electrodes, miniaturized design, and reduced electrode spacing improved the sensor response to BOD. In the laboratory test that simulated applications in the field, the MEC-type BOD sensor with EAB as the sensing element had high sensitivity, parallelism and reproducibility. It distinguished BOD concentration variations as small as 10 mg/L with a response time within 5 min, and the data gave a significant fit in the two concentration ranges of 10–50 and 50–500 mg/L to accurately determine the BOD concentration in water samples. In an actual monitoring trial as a warning system for wastewater BOD, the finished MEC sensing system was quickly assembled and started up in the field with no additional cost. More importantly, the MEC sensors activated in different ways all responded quickly, with response times less than 1 min, to changes in the concentrations of organic materials in the influent water. Although there is still a lack of repeatable data from field monitoring, the results at this stage have fully demonstrated that the MEC-type BOD sensor designed in this study has the capabilities of rapid deployment, fast response and online monitoring, showing great potential for engineering applications and commercialization.

Credit author statement

Ziyuan Wang: Investigation, Formal analysis, Methodology, Software. Chengmei Liao: Formal analysis, Methodology, Investigation and Writing. Zihan Zhong: Methodology and Discussion. Siyan Liu: Experiment and Methodology. Ming Li: Resources, Validation. Xin Wang: Designed the project, Designed the experiments and Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.114533.

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