

# A single-chamber microbial fuel cell as an alternative biosensor for continuous and onsite determination of bod in wastewater

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**Abstract.** The two main pollution parameters, BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand), are crucial factors in assessing water quality and pollution levels. Currently, COD can be measured using sensor devices, while BOD relies on the activity of microorganisms. Traditionally, the quantification of biologically oxidizable organic carbon involves measuring oxygen consumption over a five-day period, commonly known as the BOD<sub>5</sub> test. However, the BOD<sub>5</sub> test has several disadvantages, such as its time-consuming nature, unsuitability for process control, and the requirement for highly skilled samplers. It was hypothesized that the output of a single-chamber microbial fuel cell (SCMFC) with an air cathode could serve as an alternative method for measuring BOD. To validate this hypothesis, this study conducted some experiments using the model of SCMFC. When artificial wastewater, utilizing sodium acetate as fuel, was employed, a strong linear correlation ( $R^2 > 0.99$ ) between the total charge transferred and BOD<sub>5</sub> concentration was confirmed. Additionally, the linear relationship was also investigated for real domestic wastewater. This relationship was also examined for real domestic wastewater, resulting in a combined correlation with an  $R^2$  value exceeding 0.98. Until now, research on biosensors (particularly SCMFC-based biosensors) in Vietnam has been relatively new and not extensively conducted. The results of this study could provide a solid foundation for the development of continuous and onsite BOD sensors to monitor BOD concentrations in wastewater streams.

**Keywords:** BOD, Single chamber microbial fuel cell, voltage, biosensor, wastewater

**Classification number:** 3.3.3, 3.4.2.

## 1. INTRODUCTION

Microbial Fuel Cells (MFCs) have been developed as an alternative sensor device for directly and continuously measuring BOD in wastewater [1]. The basic structure of an MFC consists of a pair of electrodes (cathode/anode) connected by an external resistor [2]. Bacteria with exoelectrogenic capabilities on the anode can oxidize organic compounds in wastewater,

with electrons transferred to the cathode through an electric circuit [3, 4]. Protons ( $H^+$ ) generated at the anode move to the cathode to combine with electrons that have traversed the circuit, completing an electric circuit. Thus, MFCs generate electrical energy through exoelectrogenic bacterial groups consuming organic compounds. The variation in electrical energy produced and the amount of consumed BOD indicates that MFCs can be used as a sensor device to identify and quantify the BOD parameter. Typically, the construction of MFCs includes two basic types: single-chamber and two-chamber. For the two-chamber type, the anode chamber contains an anaerobic environment, while the cathode chamber contains an aerobic environment. The two chambers are separated by an ion-selective membrane such as Nafion [5, 6], either cations or anions depending on the focus of the research on the reaction of which chamber. In the case of the single-chamber type, the cathode chamber is directly exposed to  $O_2$  from the air (Figure 1). This is also one of the key advantages of the single-chamber MFC (SCMFC) over the double-chamber type, as it eliminates the need for air bubbling at the cathode, reducing energy consumption costs.

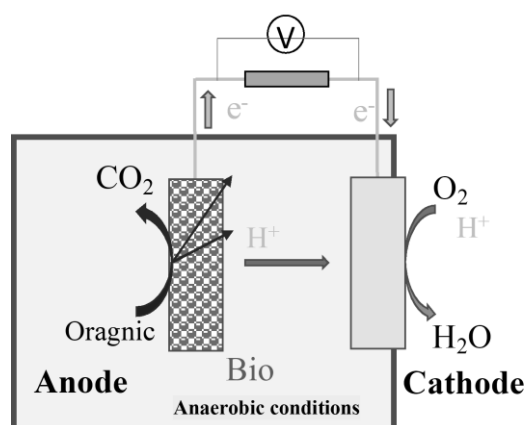


Figure 1. Scheme of a Single chamber Microbial Fuel Cells (SCMFC).

Forty years ago, Karube *et al.*, first proposed the use of Microbial Fuel Cells (MFCs) as BOD sensors [7]. *Clostridium butyricum* bacteria were immobilized on the anode electrode. Operational results with an organic substrate (glucose–glutamic acid solution) demonstrated a linear relationship between the generated current from the MFC and BOD concentration. This affirmed the feasibility of BOD sensors based on MFCs. Subsequently, various types of MFC-based BOD sensors with different strains of microorganisms were cultured and experimentally investigated [8, 9]. Compared to conventional biological sensors, MFC-based biological sensors use directly measured current or voltage as output signals [10], making them more convenient for processing and display. Moreover, they can be designed and applied in remote areas due to their self-powering capability. Despite significant achievements in research, practical applications of MFC-based BOD sensors are limited due to their relatively long adaptation times and high-tech equipment requirements.

Up to the present, research on biological sensors in general and specifically MFC-based biological sensors in Vietnam is still relatively novel and has not been extensively explored. Focusing solely on MFC sensing devices, at the national biology conference, Dr. Pham The Hai took initial steps in developing MFCs to enrich Fe bacteria for detecting Fe and Mn in water [11]. Building on these research findings, Mr. Nong Minh Tuan investigated the selection of

suitable microorganisms to enrich the electroactive microbial community in MFCs, conducting initial experiments with a simulated wastewater solution in the laboratory [12]. However, these fundamental studies primarily concentrate on the diversity of microbial communities on MFC electrodes, with limited subsequent research to test and apply MFCs in environmental fields, including biological BOD sensors.

The BOD<sub>5</sub> test has been widely used over an extended period to determine BOD in wastewater. The primary limitation of this method is its time-consuming nature, requiring highly specialized personnel for sampling, hindering the achievement of continuous water quality control. The recognition and quantification of BOD are commonly carried out through the BOD<sub>5</sub> test, posing considerable challenges in water quality control and operational management at wastewater treatment plants. BOD sensors based on MFC present an opportunity to liberate BOD parameter measurements from the confines of the laboratory. Moreover, rapid, accurate, and continuous BOD quantification has the potential to optimize wastewater treatment processes based on the input water quality and wastewater conditions throughout the operation. Therefore, applying a SCMFC model as BOD sensors serves as a foundational step in advancing research on biological sensors in water and wastewater treatment.

In this study, a series of experiments were performed by utilizing the SCMFC model. Our primary research objectives were twofold: firstly, to establish a correlation between the total charge and BOD<sub>5</sub> levels in artificial wastewater samples, and secondly, to validate the accuracy and reliability of the method by examining real wastewater samples.

## **2. MATERIALS AND METHODS**

### **2.1. SCMFC reactor and operation**

The SCMFC of this study is rectangular, with a total volume ( $V_{\text{cell}}$ ) of 220 mL, resulting in a total anodic volume of 25 mL. As can be seen in *Figure 2*, the fuel cell consists of an anode and an air-cathode, separated by a Nafion membrane with a working area of 5 cm<sup>2</sup>. Both electrodes are used carbon cloth (AA304ZS, Toyobo Co. Ltd, Japan) with an area of 8 cm<sup>2</sup> per electrode. The anode electrode was placed on one side of the cylinder (anode chamber) under anaerobic conditions and was connected via titanium wires. A small quantity of anaerobic sludge, obtained from a laboratory-scale up-flow anaerobic sludge blanket (UASB) reactor [13], was inoculated on the surface of the electrode, functioning as a bio-anode. The cathode electrode was prepared using the rolling method, as described in previous studies [14, 15]. It is placed on the open side of the plastic chamber, featuring a conductive oxygen gas diffusion layer (PTFE suspension with AC/PTFE mass ratios of six) facing the air, and an AC catalyst layer (1500 m<sup>2</sup>/g, Xinsen Carbon Co. Ltd., Fujian, China) facing the substrate medium. An external resistor is placed between the two electrodes to measure the current generated in the circuit by recording voltage using a digital multimeter (FlEPow; Levin Japan). Previous studies have highlighted the influence of external resistance on the SCMFC's performance [16]. Therefore, in this study, two specific resistance values were selected: 200  $\Omega$  for the enrichment period under continuous mode and 500  $\Omega$  for batch experiments, aiming to investigate the correlation between total charge and BOD<sub>5</sub> concentration.

The anode chamber was filled with the substrate medium and deoxidized through a nitrogen purge. The experiments were performed on synthetic wastewater with sodium acetate as a source of BOD<sub>5</sub> and on domestic wastewater samples collected directly from residential areas in Ho Chi Minh City. The substrate medium's pH was adjusted to approximately 6.5-7.5 before entering

the SCMFC using a phosphorus buffer. For the synthetic wastewater, in addition to sodium acetate as the main component, there were supplementary additions: (1) trace elements including  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (7 mg/L),  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (1.7 mg/L),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (1.5 mg/L),  $\text{HBO}_3$  (0.6 mg/L),  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (4.2 mg/L),  $\text{NiCl}_2 \cdot 4\text{H}_2\text{O}$  (0.4 mg/L),  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  (0.27 mg/L), and  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (0.25 mg/L); (2) Minerals including  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (4.0 mg/L),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (1.5 mg/L),  $\text{KCl}$  (3.0 mg/L); and (3) ammonia  $\text{NH}_4^+$  (2.0 mg/L).

The SCMFC was conducted in two consecutive phases: enrichment and batch experiments. In the enrichment phase, acetate substrate (35.7 nM) was used under continuous mode to establish stable operation before conducting measurements in batch experiments. During the batch experiment phase, initial measurements for artificial wastewater were taken with varying amounts of sodium acetate, and then they were applied to two types of domestic wastewater. Each batch experiment was conducted at a specific  $\text{BOD}_5$  value, and this process was repeated three times. Analysis of liquid samples at the beginning and end of each batch operation was performed, and  $\text{BOD}_5$  and COD measurements were carried out following international standards (APHA 1999).

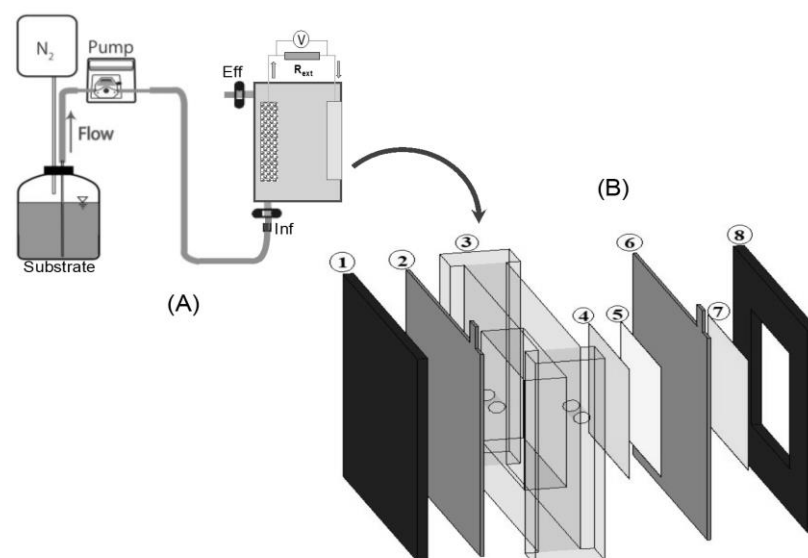


Figure 2. Scheme of Single Chamber Microbial Fuel Cells used this study. (A) SCMFC (Influent tank, SCMFC model,  $R_{ext}$  external resistance). (B) Details of SCMFC configuration – 1, 3, 8: SCMFC body; 2: Carbon cloth (anode); 4: Nafion (ion-selection membrane); 5: Catalyst layer; 6: Carbon cloth (cathode) 7: Oxygen diffusion (PTFE).

## 2.2. Analysis and calculations

The voltage measured by a was converted to current according to Ohm's law. Coulombs, expressed as current  $\times$  time, were calculated by integrating the current over the time from the start point of the experiment to the cutoff potential. Coulombic efficiency is defined as the ratio of actual coulombs of charge delivered to the anode to the total coulombs of charge generated, assuming that the total COD is being converted to biogenic electricity with a theoretical ratio of 4 mol of electrons/mol COD. The actual coulombs obtained were determined by integrating the

current over time. Therefore, Eq. (1) was used to calculate the coulombic efficiency for the SCMFC operating in fed-batch mode, measured over time  $t$ .

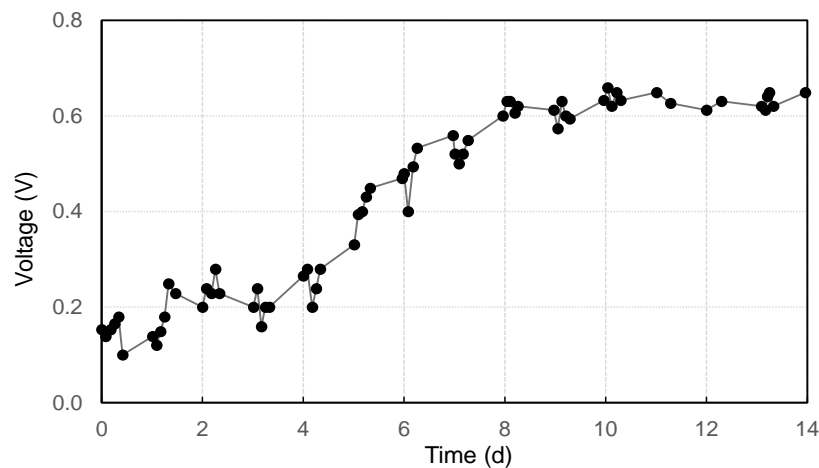
$$CE = \frac{M \times \sum I \Delta t}{4 \times \Delta COD \times V_{an} \times F} \quad (1)$$

where  $CE$  is coulombic efficiency,  $I$  is the current (A),  $t$  is time (s),  $M$  is the molecular weight of oxygen (32),  $V_{an}$  is the total anodic volume (L),  $F$  is Faraday's constant (96485.3 C/mol-electrons) and  $\Delta COD$  (g/L) is the variation in COD during the experiment.

### 3. RESULTS AND DISCUSSION

#### 3.1. Enrichment

The anode of SCMFC was inoculated with diluted anaerobic sludge. The enrichment was sustained by artificial wastewater, comprising sodium acetate substrate at a concentration of 35.7 mM, trace elements (1 mL/L), and minerals (0.5 mL/L) [17]. *Figure 3* shows the variation in the output voltage of a SCMFC over time under an external load of 200  $\Omega$ , converting sodium acetate into electrical energy. The substrate medium continuously filled up the SCMFC at a rate of 0.224 mL/s to observe the system's response, including the initial start-up phases and the establishment of steady-state conditions. In the initial four days, an electric current was generated in the circuit, and the measured voltage increased gradually from 0.18 V to 0.28 V. However, starting from the fifth day, the output increases more rapidly and stabilizes at the level of 0.6 - 0.65 V. The increasing voltage over time indicates enhanced electrochemical activity of the bacteria, adapting to the environment. This also suggests that the enrichment for electrochemically active bacteria was completed in approximately eight days.



*Figure 3.* Various voltage of output voltage of SCMFC with time when fed with continuous mode of sodium acetate solution.

#### 3.2. Performance of batch experiments

After the completion of the enrichment process, the SCMFC operated in sequential batches to establish the correlation between the electrical charge across the circuit and the corresponding

BOD<sub>5</sub> concentration. The artificial wastewater was carefully regulated, with sodium acetate as the main component, supplemented with minerals, trace elements, and a phosphorous buffer to maintain the pH within the model at approximately 6.5 - 7.5. Five different values of BOD<sub>5</sub> concentrations (81, 159, 318, 417, 636 mg/L) were used, with each BOD<sub>5</sub> value corresponding to one batch and repeated three times. After testing artificial wastewater samples, the SCMFC continued to process domestic wastewater with BOD<sub>5</sub> concentrations of 178 and 253 mg/L, under the same experimental conditions as the artificial samples. Considering the BOD<sub>5</sub> value used in the enrichment and the concentration dependence of the voltage in the cell [18], an external resistance of 500 Ω was chosen for batch experiments.

Initially, SCMFC was operated with a BOD<sub>5</sub> of 318 mg/L. The voltage reached its maximum immediately after filling up the substrate into the model and then gradually decreased due to fuel consumption. When the voltage dropped to approximately 0.1 V, it was observed that the voltage no longer decreased. Continuing to maintain this condition for an additional 24 hours showed no change in voltage. In the subsequent batches, for example, at BOD<sub>5</sub> concentrations of 159 and 477 mg/L, the measured voltage exhibited tendencies like those seen at a concentration of 318 mg/L. Therefore, 0.1 V serves as the baseline voltage without substrate, related to endogenous respiration. It may also function as the cutoff potential. However, when applied to real wastewater, the voltage dropped to a lower level, reaching the potential cutoff of 0.06 V.

### 3.2.1. Evaluation of measurements with artificial media samples and real wastewater samples

Figure 4 shows the temporal variation in voltage corresponding to different concentrations of sodium acetate. The maximum voltage output manifested as near-instantaneous peaks upon sample insertion, gradually decreasing to the established cutoff potential of 0.1 V. Within the range of low BOD<sub>5</sub> concentrations (<320 mg/L), the voltage demonstrated a proportional relationship with BOD<sub>5</sub> concentration. However, at higher BOD<sub>5</sub> levels, the voltage reached its maximum at almost a consistent value. These findings align with prior studies on SCMFC employing air-cathodes [16, 19, 20], while total charge was correlated with BOD concentration up to 520 mg/L in a two-chambered system [21]. The broader dynamic range observed in the SCMFC with an air-cathode suggests enhanced MFC based on BOD sensor performance achievable through cathode selection. Additionally, the correlation between max current output and BOD<sub>5</sub> concentration implies the potential for continuous, real-time BOD measurements in situ.

This research also investigates variations in the voltage vs time curve during batch experiments employing real wastewater. Notably, the voltage-time curves exhibited slightly distinct patterns between artificial and real wastewater. Additionally, the max output voltage and the cutoff potential were lower than when sodium acetate served as the carbon source (*Figure 4*). This discrepancy was anticipated due to the microbial culture's acclimatization to sodium acetate. Tardy *et al.*, 2021 suggested that a "two-step" shape of curves was observed with domestic wastewater characterized by high BOD concentrations [20]. The first step corresponds to the biodegradation of soluble, readily biodegradable compounds, while the second step involves the biodegradation of suspended, slowly biodegradable compounds. Further research is necessary to elucidate the aforementioned identification. This can potentially lead to the determination of the ratio between soluble readily biodegradable and suspended slowly biodegradable substrates.

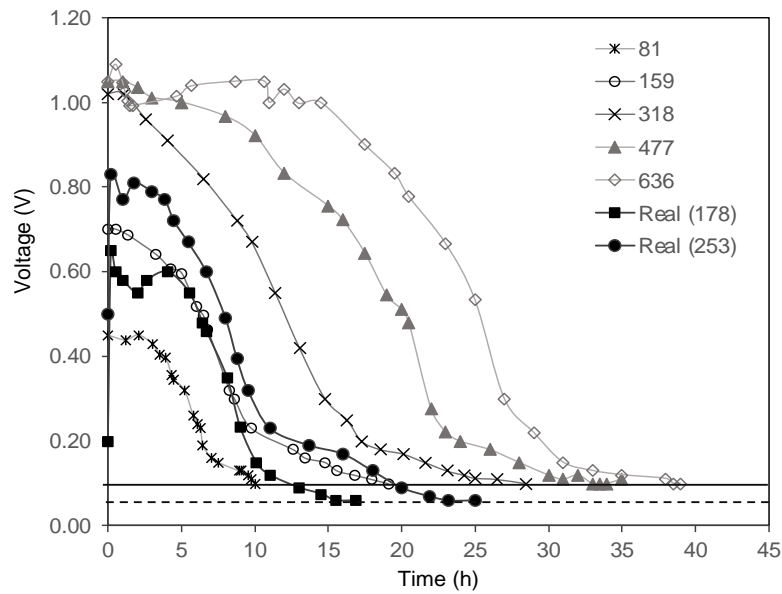


Figure 4. Voltage vs time curves for artificial wastewater (with different concentrations of sodium acetate) and domestic wastewater. Solid line: 0.1 V cutoff potential applied for artificial wastewater; Dashed line: 0.07 V cutoff potential applied for domestic wastewater.

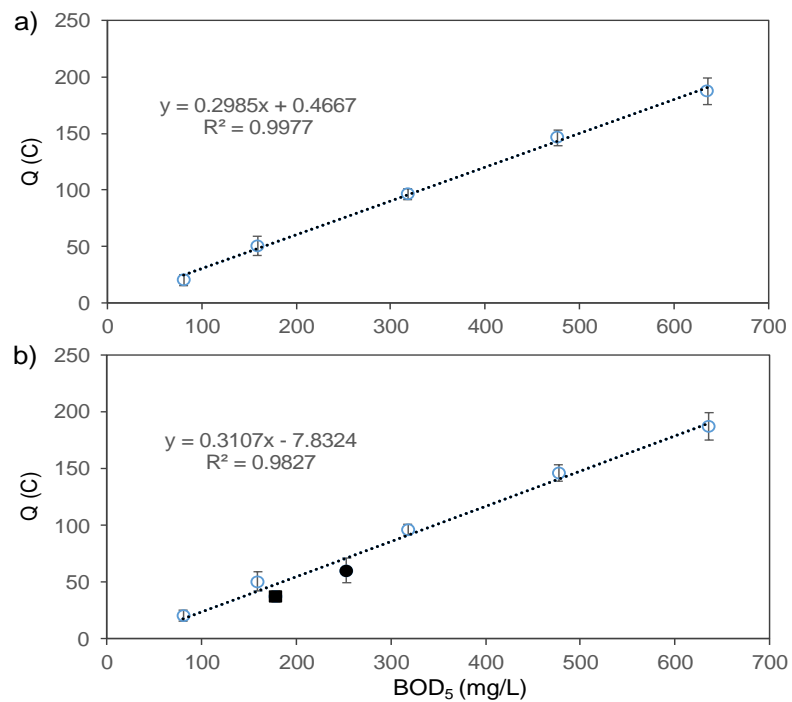


Figure 5. a) Linear correlation of the total charge values vs BOD<sub>5</sub> concentrations for artificial wastewater (with different amounts of sodium acetate)  
 b) Combined linear correlation of the total charge values vs BOD<sub>5</sub> concentrations for artificial wastewater and real wastewater.

In Figure 5a, a good linear correlation is observed between the calculated total charge values and the BOD<sub>5</sub> values of the artificial samples. The coefficient of determination ( $R^2$ ) is higher than 0.99. Despite a slight reduction in the output voltage for real wastewater compared to measurements derived from sodium acetate, employing a unified calibration approach for both artificial and real wastewater is recommended (Figure 5b). Notably, the R-squared ( $R^2$ ) values for these consolidated linear correlations consistently exceed 0.98, attesting to the remarkable precision and reliability of this analytical method. This outcome underscores the method's ability to accurately assess two types of wastewater through the use of a singular calibration line, highlighting its robustness and applicability in diverse environmental monitoring scenarios.

### 3.3.2. Coulombic efficiency

Achieving high coulombic efficiency is imperative for the optimal operation of an MFC-based biosensor, especially in the presence of alternative electron acceptors like nitrate and oxygen with higher redox potentials, which can potentially diminish the signal and lead to a decline in overall biosensor performance. In this study, the utilization of artificial wastewater as a fuel in the small-volume SCMFC biosensor may yield the best performance in terms of response time and coulombic efficiency. Employing a nitrogen purge to deoxygenate the substrate was adopted as a strategy to limit dissolved oxygen (DO) in water [5, 22]. However, the coulombic efficiencies of this SCMFC model were not exceptionally high, registering values of 44 %, 42.8 %, 41.4 %, 41.2 %, and 40.1 % for BOD<sub>5</sub> concentrations ranging from 81 mg/L to 636 mg/L in artificial wastewater. For real wastewater, the coulombic efficiency was even lower at 36 %, corresponding to an influent BOD<sub>5</sub> of 178 mg/L. The observed lower efficiencies may be attributed to the entry of oxygen into the reactor, creating a competition between DO and the anode for the electrons released from the substrate. Notably, in systems with an air cathode, substantial oxygen diffusion into the anode chamber is anticipated, even with a polymeric membrane like Nafion [23]. Furthermore, compared with previous studies, the initial concentration of substrates also impacts Coulombic efficiency [16], [19]. At low BOD<sub>5</sub> values, the short consumption time of BOD helps mitigate the potential influences on reaction efficiency. Given these findings, it becomes particularly intriguing to explore the effects of inhibitors of aerobic respiration on the performance of the SCMFC-based biosensor.

The limitations of the traditional measurement method prompt the exploration of alternative approaches, given that the current standard demands an extensive five-day timeframe and skilled personnel for precision. Additionally, the setup duration of two hours renders the traditional method less practical for routine testing of a limited number of samples, especially in remote locations. Recognizing the significance of rapid and accurate BOD monitoring in the water industry, environmental treatment processes, and regulatory frameworks, there arises a necessity for a new measurement method. The SCMFC-based biosensor emerges as a promising alternative to the conventional BOD<sub>5</sub> test. The stable bacterial community on the anode surface during the stable stage of the MFC, coupled with the proportional influence of substrate concentration on the metabolic rate of electrochemically active bacteria, makes the MFC-type BOD sensor a viable option. Evaluating the performance of the SCMFC based on a BOD sensor using varying concentrations of sodium acetate solution and real wastewater in this study demonstrated a strong linear correlation between BOD concentration and transferred charge ( $>0.98$ ), showcasing its applicability under specific conditions. This innovation holds the potential for revolutionizing BOD measurements, providing a more efficient and timely approach in contrast to the limitations posed by the traditional methodology.



## 4. CONCLUSIONS

This study demonstrated that an SCMFC could be used as a biosensor for BOD determination. A strong linear relationship between the total charge transferred and BOD concentration of sodium acetate in a range from 81 to 636 mg/L, with a coefficient of determination ( $R^2$ ) reaching 0.9977, was found. The correlation remains strong even when tested with real domestic wastewater having BOD concentrations of 178 and 253 mg/L. It is suggested that the potential for a combined correlation for both artificial and real wastewater. The  $R^2$  value of this linear correlation is higher than 0.98. In terms of future research, despite the promising results, the coulombic efficiency remains less than expected. Beyond factors related to the model's structure and operation, it is crucial to assess the impact of inhibitors on microbial activity and the performance of the SCMFC-based biosensor by analyzing the microbial community on the anode. Additionally, as the SCMFC has primarily been applied to municipal wastewater rich in easily biodegradable organics, further studies are needed to develop models applicable to various industrial wastewater types. These efforts aim to enhance the overall performance and versatility of the SCMFC-based biosensor for broader practical applications.

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**CRedit authorship contribution statement.** Dinh Thi Thu Ha: Methodology, Investigation, Formal analysis, Writing & Editing, Funding acquisition, Supervision. Pham Ngoc Phat: Formal analysis.

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