Journal of Environmental Management 276 (2020) 111279

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: http://www.elsevier.com/locate/jenvman

Research article

Performance of mediator-less double chamber microbial fuel cell-based biosensor for measuring biological chemical oxygen

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ARTICLE INFO

Keywords:
Bioelectricity
Biosensor
Wastewater management
Nafion membrane
Biochemical oxygen demand

ABSTRACT

Recently, the microbial fuel cell-based biosensor has been considered as an attractive technology for measuring wastewater quality such as biochemical oxygen demand (BOD). In this study, a mediator-less double compartment MFC based biosensor utilizing carbon felt as an anode electrode and inoculated with mixed culture was developed to improve the real application of a rapid BOD detection. This study aims to: (i) establish the effect of the operating conditions (i.e., pH, external resistance, fuel feeding rate) on MFC performance; (ii) investigate the correlation between biochemical oxygen demand (BOD) and signal output, and (iii) evaluate the operational stability of the biosensor. The presented result reveals that the maximum current and power production was obtained while 100 mM NaCl and 50 mM Phosphate buffer saline was used as a catholyte solution, neutral pH condition of media and fuel feeding rate at 0.3 mL min^{-1}. Notably, a wider range of BOD concentration up to 300 mg L^{-1} can be obtained with the voltage output ($R^2 > 0.9901$). Stable and steady power was produced by running MFC in 30 days when cells operated at 1000 Ω external resistance. Our research has some competition with the previous double chamber MFC in the upper limit of BOD detection. This results might help to increase the real application of MFC based BOD biosensor in real-time measurement.

1. Introduction

Biochemical oxygen demand (BOD) is one of the most commonly-used indicators for assessing wastewater quality. The traditional BOD method takes 5–7 days and also depends on labour skill for expected accuracy (Li et al., 2016; APHA, 1992). Therefore, it is not reasonable for online monitoring wastewater quality. Microbial fuel cell-based biosensor has been regarded as a feasible alternative technology to determine the BOD parameter.

Microbial fuel cell (MFC) has been considering an alternative to deal with the two contemporary issues: environmental pollution and exhausting fossil fuel. MFC is an electrochemical technology which possibly converts chemical energy to electricity by using catalytic bacteria to oxidize the organic matters (Do et al., 2020; Feng and Harper, 2013). The basic components of MFC include anode and cathode chambers which separated by a proton exchange membrane (PEM). In the anode chamber, the substrate was oxidized by micro-organism in anaerobic condition and this process generates electrons and protons. Electrons are then transferred from the anode electrode to the cathode chamber via an external circuit. On the other hand, protons are transported through the PEM to the cathode reactor where they are combined with electron acceptor (e.g., O_2, KMn O_4, metal, nitrate) to form water (Wang et al., 2019). MFC has many advantages compared to the traditional technologies including energy-saving, effective pollutant remediation and, particularly, it can be applied in remote areas where electric is insufficient. Thanks to those benefits, several applications of MFC have been developed such as bioelectricity and biohydrogen production, wastewater treatment and biosensor (Sivasankar et al., 2019).

The microbial fuel cell can be operated as a biosensor, in which anode biofilm play a role as a biological sensing element while

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https://doi.org/10.1016/j.jenvman.2020.111279
Received 29 June 2020; Received in revised form 18 August 2020; Accepted 19 August 2020
Available online 3 September 2020

electrodes have a function of a transducer. The microorganisms in the anode chamber can sense the presence, or any change of the input analyse target, then give the response to the output signal (Corbella and Puigagut, 2018; Do et al., 2020). Typically, BOD concentration can be observed with the current or voltage output within 5 min–10 h. The most benefit of this MFC technology is that no extra transducer needs to recognize and translate the input signal to a measurable signal since the anode compartment plays a role as a transducer. Therefore, microbial fuel cell biosensor can be considered as a promising and simple tool that fully meets the requirement for a long-term BOD biosensor.

The first MFC based BOD biosensor was investigated by Karube et al. (1977), in which *Closditrun butyricum* was immobilized on the electrode. This study showed a correlation between BOD and current output. Another study of BOD biosensor was conducted by Kim et al. (2003) utilizing wastewater. Up to 206 ppm BOD concentration was detected with coulomb. In a later study, a MFC biosensor which utilized a mix of six bacteria strains to detect BOD was developed by Hsieh and Chung (2014). The described MFC biosensor successfully measured BOD concentration below 240 mg L^{-1} in real wastewater sample.

Recently, Yamashita et al. (2016) developed a novel bio-electrochemical which showed the correlations between the current output and BOD concentration up to 250 mg L^{-1} with logarithmic $R^2 >$ 0.9. This biosensor can apply in a remote area of natural water environments. A novel single-chamber MFC-based BOD biosensor was investigated in real wastewater by Kharkwal et al. (2017). Researchers help to reduce the electrode material cost by utilizing MnO_2 as a catalyst in the cathode compartment for oxygen reduction. This research illustrated the good correlation between BOD concentration and the signal output. Other researchers have been improved the architecture of the MFC by combination with other physical, chemical, and biological processes such as a multi anode/cathode MFC, a new sediment MFC was explored by Jiang et al. (2010) and Chen et al. (2012), respectively. This integration has some benefits from decreasing the distance between the electrode, which can help to reduce internal resistance. However, the author also revealed that these integrate MFC is more expensive than traditional MFC in building and have some troubles in maintenance.

There are some crucial impacts affect the efficiency of MFC biosensor performance and energy generation including architecture factors, operating conditions, and biological elements (Jung and Pandit, 2019; Choi and Ahn., 2013). Among these influence factors, the environmental parameter such as pH, temperature, electron donor, electron acceptor, fuel feeding rate, conductivity is the most vital element which affects to the accuracy and precision of the MFC (Aghababaie et al., 2015). Besides, material and configuration of the reactor, electrode and membrane are also the crucial impacts that must be considered, since it influences the cost and the performance of the MFC biosensor system.

Although there has a lot of improvement in MFC-based biosensors over the past decade, this technology still has a limitation in its real application and scale-up. The present study is, therefore, to establish a simple compact MFC-based biosensor for high stability and rapid detection BOD. Carbon felt which has high porosity, high electrical conductivity, and low in cost was utilized at anode electrode. NaCl and Phosphate buffer solution (PBS) was exploited to the catholyte solution to increase the ionic strength to get a better power generation. Furthermore, the effects of critical operating parameters (pH, flow rate, external resistance) were investigated to optimize MFC performance. The stability of the sensor was carried out. The relationship between BOD and the potential output of MFC was illustrated. Lastly, the structure of the anode biofilm was presented in this study. These present results could help improve the real-world microbial fuel cell-based biosensor applications.

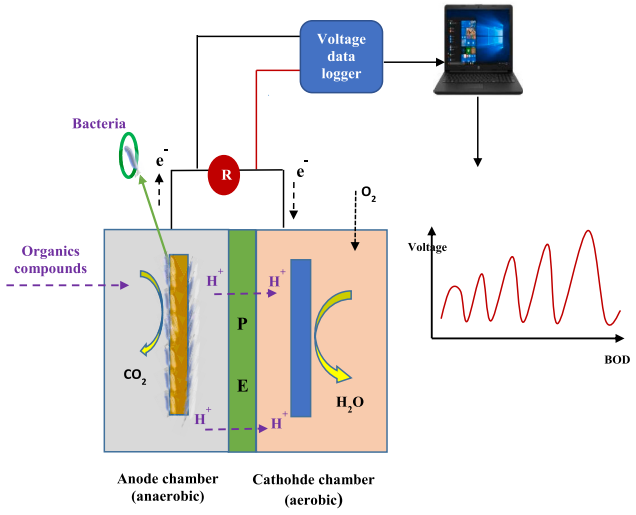

Fig. 1. Microbial fuel cell based biosensor system.

2. Materials and methods

2.1. MFC configuration

The architecture of an MFC biosensor was a traditional double-compartment in rectangular form. Both reactors (anode and cathode) were made from plexiglass with the working volumes being 300 ml and 400 ml, respectively. For anode utilization, the Carbon Felt CF 120 was 0.3 cm in diameter and 0.3 cm in thick (The Fuel cell store, Texas, USA). A carbon fiber brush was employed for the cathode electrode where the length and the diameter of the brush were 25 mm and 30 mm, respectively. Nafion 117 was selected as a proton exchange membrane located between electrodes. Electrodes were connected through a resistor by a platinum wire. The anode compartment was maintained in anaerobic conditions by purring nitrogen gas. Air was supplied continuously into the catholyte solution to provide O_2 needed for the electrochemical reaction. Fig. 1 below illustrates the configuration of the MFC system.

2.2. Synthetic wastewater composition

In this study, we used glucose as an organic carbon source, ammonium chloride, and potassium phosphate which containing nitrogen and phosphorus were used as a source of nutrients. The components of the synthetic wastewater were 300 mg L^{-1} glucose, 4.6 mg L^{-1} KH_2PO_4, 20 mg L^{-1} NH_4Cl, 5.4 mg L^{-1} $MgSO_4.7\ H_2O$, 0.4 mg L^{-1} $CaCl_2$. 2 H_2O, 32 mg L^{-1} yeast and 0.61 ml of trace nutrients. The trace nutrient solution includes 0.275 mg $L^{-1}MnCl_2{\cdot}7H_2O$, 0.44 mg $L^{-1}ZnSO_4{\cdot}7H_2O$, 1.45 mg L^{-1} $FeCl_3$, 0.391 mg $L^{-1}CuSO_4{\cdot}5H_2O$, 0.42 mg L^{-1} $CoCl_2{\cdot}6H_2O$. The synthetic solution was controlled with nitrogen gas in 15–30 min before feeding it to the reactor. The operating temperature of the biosensor system was kept at room temperature ranging from 25 to 33 °C.

2.3. Enrichment and immobilization

The MFC biosensor was exploited to cultivate electrochemically bacteria utilizing artificial wastewater as the electron donor and sludge as the bacterial source. The anaerobic sludge in the present study was gathered from a domestic wastewater treatment plant in Sydney (Cronulla wastewater treatment plant) and cultured for four months before starting the experiments. 300 ml of municipal wastewater including 100 ml sludge was fed into the anode chamber in batch mode under an external resistance of 1000 Ω. After approximately 4–6 weeks of operation, the potential gained a steady-state, this meant that the biofilm has been attached to the anode surface and considered mature enough for

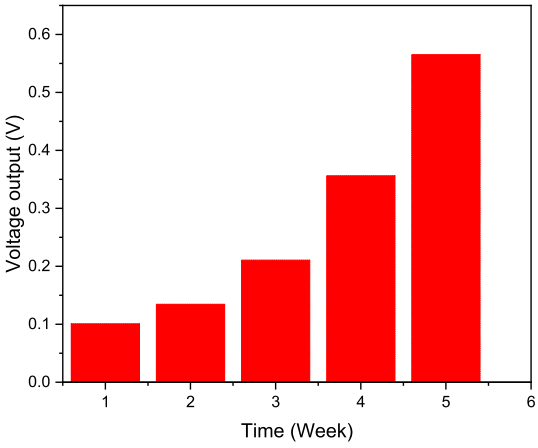

Fig. 2. Voltage output of the start-up time.

operating.

2.4. Experiment process

The artificial wastewater was added into the anode chamber by a peristaltic pump at a fuel rate of 0.3–1.0 mL min^{-1}. The oxidant was flushed continuously into the cathode chamber at different rates of 20–100 mL min-1. The Deionized water, NaCl, and phosphate-buffered saline (PBS) with diverse concentrations ranging from 50 mM to 100 mM were introduced to cathode solution to get the optimization. To establish the polarization and power curve of the MFC biosensor at the stable stage, different external resistors from 50 to 47000 Ω were expressed. To create the relation between BOD and the signal output (voltage or current), a diverse range of BOD concentration from 50 to 300 mg L^{-1} was set up. The MFC based biosensor' stability was estimated by running the system at fixed BOD concentration at 200 mg L^{-1} for 30 days. The structure of the matured biofilm was carried out by running scanning electron microscopy (SEM) (Zeiss Supra 55VP, Carl Zeiss AG).

2.5. Analysis method

The potential output U between electrodes was measured continuously every10 min by utilizing a voltage data logger (VOLT 101 A Madge Tech) connected to a PC through a software. The current I (mA) was calculated according to Ohm law,

$$I = \frac{U}{R} \quad (1)$$

The current density *i*, the power output P, the power density *p* calculated by the following equation.

$$Power\ output:\ P = U^{*}I \quad (2)$$

$$\text{Power density (p)}: p = \frac{P}{A} \quad (3)$$

$$\text{The current density (i)}: i = \frac{U}{RA} \quad (4)$$

U: Voltage (mV)
R: external resistance (Ω)
I: Current (mA)
A: surface area of the anode material (m^2)

During the experiment, the pH parameter of the MFC system was checked every day by a pH meter (Hach Company, model no. HQ40d). The dissolved oxygen was control by a DO meter (OM-51, Horiba, Tokyo, Japan).

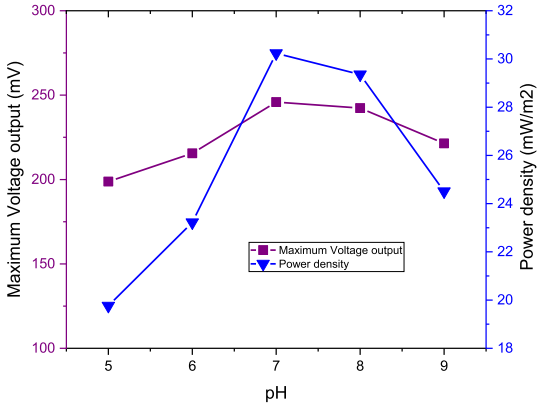

Fig. 3. The effct of pH on power generation of MFC-based BOD sensor.

3. Results and discussion

3.1. Enrichment efficiency

As described at the previous pare, MFC biosensor was inoculated by utilizing activated sludge and fed with artificial wastewater. The open-circuit voltage of the biosensor (external resistance $R_{ext} = 0$) was nearly 0.6 V after 10–12 h. Fig. 2 illustrates the voltage output collected from the enrichment process. At the earlier stage of the enrichment process, the voltage output of the MFC biosensor rise slowly, however, it gradually increases during the time. After nearly 5 weeks, the potential difference between electrodes can gain the maximum value of 0.56 V, and no further increase in voltage output was detected which illustrated that the anode biofilm was developed and matured enough for operating MFC biosensor. After the enrichment of the fuel cell was finished, the performance of the MFC was established.

3.2. The influence of anodic pH on the operating of MFC

Anodic pH is a vital operating parameter that directly affects not only the activity of microorganisms but also the kinetic of protons transportation through the membrane (Jia et al., 2014). In current work, the performance of the biosensor was examined as the function of potential output generation at diverse pH from 6.0 to 9.0. The microbial fuel cell was operated at 20–30 °C through the external resistance R 1000 Ω. Wastewater with BOD 200 mg L^{-1} was utilized during the experiment.

As can be seen from Fig. 3, the highest value of the voltage output was 245.9 mV at pH 7. Interestingly, there were a 19.15% and 9.96% reduction in voltage at pH value 5 and 9 compared to that at pH 7. However, only slightly lower in voltage at pH 8 (242.3 mV). The similar trend can be observed in the power density. The maximum power density was obtained at pH 7 and lower power generated at the acidic and alkaline condition.

These results can be explained is that the lower pH of the anodic chamber hinders electrogenic bacteria's activity, resulting in inhibition in the voltage generation. The higher voltage creation was obtained at neutral pH can be explained that is the optimal condition for bacterial growth. These results agree with what previous researches have documented. Gil et al. (2003) established the influence of anodic pH on MFC performance. Authors revealed that the neutral pH (7–8) was the optimal value for a microbial consortium cultured. Ren et al. (2007) also indicated that there was a dramatic reduction in the power creation at pH in the anode compartment under 5.2, however, it was rapidly resumed when the pH increases up to 7.

3.3. The influence of catholyte solution on MFC biosensor

The cathode reactor encompasses a medium that must be aerated to

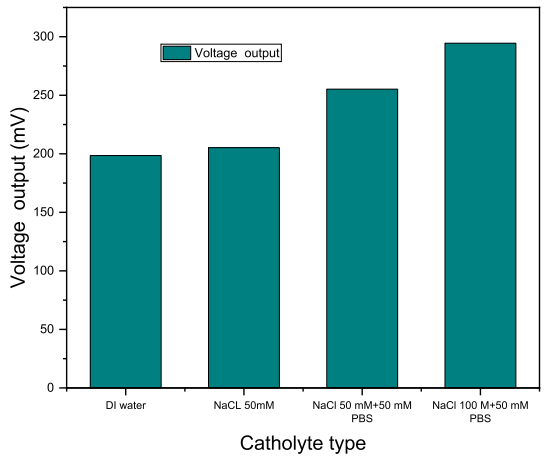

Fig. 4. The potential voltage output of the MFC biosensor with different catholyte solutions.

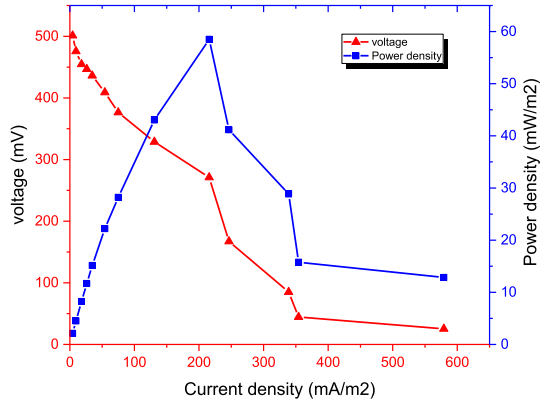

Fig. 5. Polarization and power curves of the MFC-based BOD biosensor.

supply oxygen for the cathode. Oxygen is considered the most popular electron receiver which can help to promote the power production of MFC. To observe the influence of the catholyte solution on the operating of MFC biosensor, four types of different compositions encompass deionized water, 50 mM NaCl, 50 mM NaCl +50 mM PBS, 100 mM NaCl +50 mM PBS, were examined to achieve the optimal catholyte solution in terms of the maximum power production. The double chamber MFC biosensor was run in a batch mode, and each experiment was conducted at least 3 times. External resistance R = 1000 Ω was used, and the BOD concentration of the anode was prepared at 200 mg L^{-1} during the experiment.

As can be seen from Fig. 4, the maximum voltage could reach 294.5 ± 2.45 mV for the cathode solution with 50 mM PBS and 100 mM NaCl and there was a decrease in the voltage output generation with other catholyte solution ranging from 198 mV to 275.5 mV. Higher NaCl concentration leads to higher voltage output generation in MFC biosensor. The main explanation only for this is due to the influence of the ionic strength of the catholyte solution on the voltage output generation. According to the previous study, Liu et al. (2005) examined the effect of ionic strength of electrolyte utilizing NaCl up to 300 mM and indicated that the voltage output increase with increasing the ionic strength. Huang et al. (2010) also illustrated hat the power output can significantly increase by increasing the ionic strength due to the reduction in the internal resistance of MFC. Du et al. (2007) and Feng et al. (2008) also revealed that higher conductivity of the solution leads to a decreased ohmic loss, in turns higher power production was obtained in MFC biosensor. Besides, Nam et al. (2010) also indicated that phosphate buffers are widely used in MFC research, and it has been

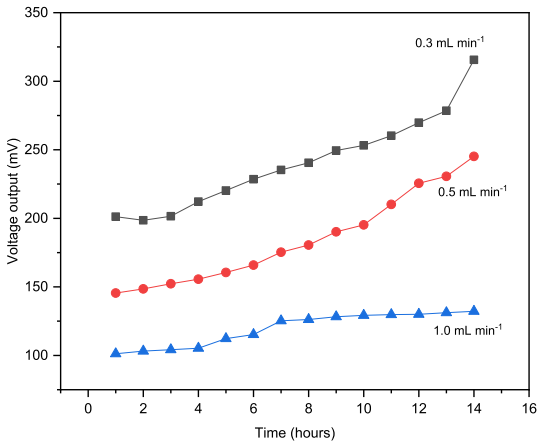

Fig. 6. The influence of different fuel feeding rate on the voltage output of the MFC system.

revealed that the power output increase with the raise of phosphate concentration within certain ranges. Therefore, 50 mM PBS and 100 mM NaCl were selected for the electrolyte during this study's experiment.

3.4. Effect of external resistance

To test the influence of the external resistance to power production of MFC, the external resistance of the MFC was setup by increasing gradually from 50 to 47000 Ω. The polarization was explored as a function of voltage output, power density, and current density. Voltage output was obtained at the stability stage of MFC. The performance of the MFC was assessed under each external resistance factor for two days.

As can be observed from Fig. 5, the voltage output of the biosensor increase when raising the external resistance. It means that there was an inverse proportion between the voltage output with current density. Nonetheless, the graph provides the variation of power density with current density. The power density was firstly increased in proportion with the current density, but then that trend changed to inversely proportional. The power density was in the 2.13–58.52 mW/m^2 range, while the current density was in the 4.25–579.22 mA/m^2 range. This present results in an agreement with some previous works.

Menicucci et al. (2006) revealed that when decreasing resistance from 6 to 0.125 kΩ the voltage generation reduce. This might be understood that operating MFC at higher resistance causes a limitation on the mass transfer, on the kinetics of the electron transportation to the anode electrode. A similar reduction trend of current production when the external resistance decreases from 50 to 10.5 Ω was obtained in a study by Aelterman (2009). In another study, Ghangrekar and Shinde (2007) showed that there was an increase in cell voltage when raising the resistance from 0 to 4000 Ω. The maximum voltage of 358 mV was observed at external resistance 4000 Ω. A similar result of cathode potential was gained at variety value of external resistance in research by Rismani-Yazdi et al. (2011).

3.5. Effect of fuel feeding rate on the power output of MFC

To explore the performance of the MFC, the fuel feeding rate and the airflow at the cathode chamber were tested. Four different flow rates of 0.3, 0.5, 0.7, 1.0 mL min^{-1} were examined for the fuel feeding rate into the anodic compartment. The experiment was carried out with the 200 mg l^{-1} BOD, external resistance 1000 Ω, phosphate buffer saline 50 mM with NaCl 100 mM as the catholyte. As can be observed from Fig. 6, the highest value of the voltage output signal was 315.5 mV at the fuel rate of 0.3 mL min^{-1}. However, there is no significant increase in the voltage signal was detected at the fuel rate of 0.7–1.0 mL min^{-1}. This indicated that anode flow rate wields effects on the MFC signal, due to mass

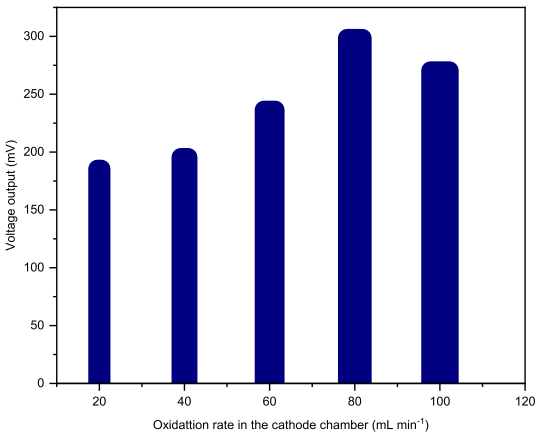

Fig. 7. Voltage output of the MFC at different oxidation rate at the cathode chamber (20–100 mL min^{-1}).

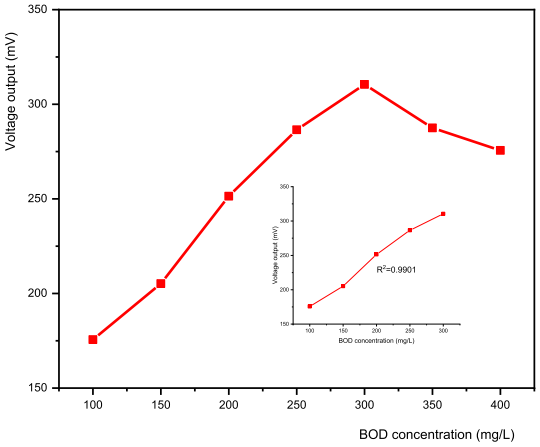

Fig. 8. Relationship between BOD concentration and voltage output of the MFC biosensor.

transfer and stability of biofilms. Normally, the power output increase with the raise of flow rate, however, Ieropoulos et al. (2010) also illustrated that if the flow rate is too high it may lead to a reduction in power density production. A reasonable can be explained is that a higher flow rate has inhibition on the microbial community development, the structure and the stability of the biofilm were not matured enough that lead to a reduction in energy creation. Therefore, this current result illustrated that the performance of the MFC was characterized by the optimal feeding rate.

In terms of the influence of the oxidant rate in the cathode reactor to the performance of MFC biosensor, four different flow of 20, 40, 60, 80, and 100 mL min^{-1} were tested while maintaining the anodic flow rate under optimized condition. Other conditions in this experiment were similar to those of the fuel rate experiment. As can be obtained from Fig. 7, the results indicated that the maximum voltage increased with the aeration rate in the range of 20–80 mL min^{-1}. However, at a higher oxidant flow rate in the cathode (100 mL min^{-1}), no significant increase in signal output was obtained. The reason might be that the oxygen reduction reaction at the cathode was inhibited by oxidant supply at a high flow rate. This outcome was consistent with another research study conducted by Gil et al. (2003).

3.6. Relationship between BOD concentration and output voltage

The MFC was operated using variable influent BOD of wastewater (100–350 mg l^{-1}) to carry out the correlation between the substrate

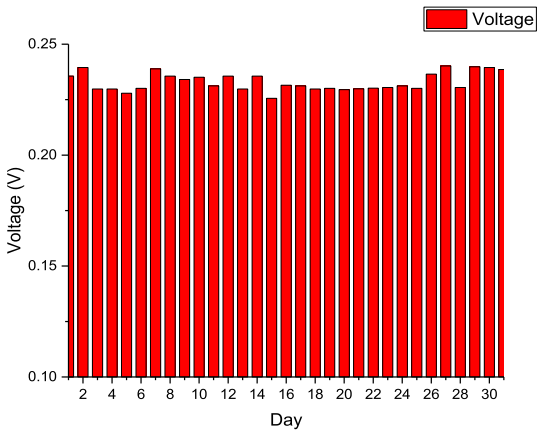

Fig. 9. The stability of MFC over time. BOD = 200 mg l^{-1}. R = 1000Ω. Catholyte: 50 mM PBS+100 mM NaCl.

concentration and the current produced. As shown in Fig. 8, a good linear relation was obtained for BOD concentration ranging from 100 to 300 mg L^{-1}. When BOD concentration exceeded 300 mg/l, no correlation was detected. The only reason for this is the metabolism of the microorganism greatly depends on the organic concentration of the electrolyte under low substrate concentrations. At the higher substrate concentration (>300 mg L^{-1} BOD), the anodic solution was saturated with electroactive bacteria. Moreover, a higher organic loading rate of the anode chamber might lead to membrane fouling, which could affect the electricity generation (Elakkiya and Matheswaran, 2013). He et al. (2015) also discovered that a higher organic loading rate could lead to volatile fatty acid (VFA) accumulation, which affects electricity generation. Apart from this, the activity of methanogenic microorganisms will increase with a higher organic loading rate, and consequently, compromise the electricity production ability of the electrogenic bacteria .

The findings in this study agrees with what previous studies have reported. The correlation between BOD concentration and coulomb generation revealed good linearity up to 206 mg L^{-1} by running an MFC in a batch mode (Kim et al., 2003). Chang et al. (2004) illustrated a linear correlation between current and BOD up to 100 mg l^{-1} by utilizing a mediator-less double chamber MFC. Nakamura et al. (2007) developed a dual-chamber MFC without adding a mediator obtained a similar trend. Yang et al. (2013) investigated a single chamber MFC with carbon felt act as anode material. A good relationship between voltage output and BOD concentration up to 120 mg L^{-1} was established. In comparison with previous analyses, the present results have some competitive beneficial due to the wider range of upper BOD measurements (up to 300 mg L^{-1}).

3.7. The stability of the MFC biosensor

Stability is one of the essential factors that must be considered when dealing with biosensors. The stability of the microbial fuel cell biosensor was carried out by running 200 mg L^{-1} BOD over 30 days (Fig. 9). The analysis was carried out in triplicate. The voltage output was stable during the testing period, and the average voltage was 233.26 mV with a standard deviation of ±3.64%. Our study was consistent with another study, namely that conducted by Kumlanghan et al. (2007), who obtained a standard deviation of ±5.5%.

3.8. Biofilm growth on the anode

The development of biofilm on the anode surface area is very important to the MFC biosensor. Yang et al. (2012) illustrated that biofilm formation has a great influence on the kinetic of transferring

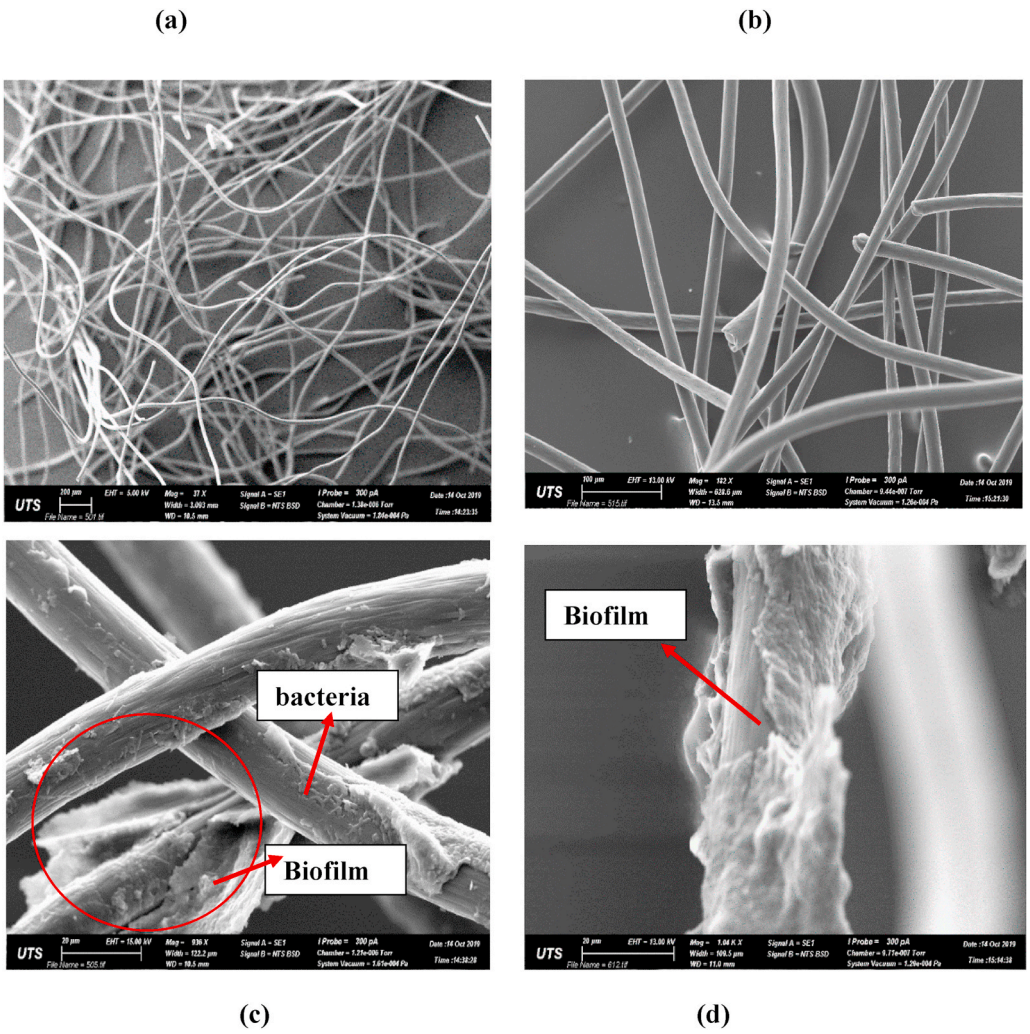

Fig. 10. SEM images on carbon felt before and after biofilm formation (a,b: new carbon felt, c,d: biofilms formation on the surface of the anode electrode).

electrons extracted from the metabolic oxidation to an electrode, consequently, effect to the energy production of the biosensor. The biofilm development depends on some environmental factors such as type of nutrient, pH, flow rate, external resistance (Toyofuku et al., 2016). The surface of the biofilm formation at the anode electrode was carried out by scanning electron microscopy (SEM) (Zeiss Supra 55VP, Carl Zeiss AG). The carbon felt was removed and cut a small piece from the anode chamber and then immediately fixed in PBS with 2.5% glutaraldehyde for 4 h. The sample was then washed three times with water and dehydrated stepwise in a series of ethanol solutions (30, 50, 70,80,90, and 100%) for 10 min and then critical –point dried with a tert-buttylethanol solution. Finally, the sample was coated with a thin gold layer and observed in a high vacuum using SEM. As can be seen in Fig. 10, the surface of the unused carbon felt was very smooth, with much straight carbon crossed over each other (Fig. 10a and b) and the biofilm attached to the anode electrode with dynamic bacteria was observed.

4. Conclusion

The study has successfully developed a mediator-less microbial fuel cell-based BOD biosensor with excellent performance, highly reliable, and stable. A good relationship between voltage output and BOD concentration up to 300 mg L^{-1}, which has some competition with this reported in previous researches. Additionally, the effect of different factors (e.g, the anodic pH, different catholyte solutions, organic matter concentration, external resistance) affecting the performance of the BOD sensor was examined. Overall, the findings help facilitate the potential application of microbial fuel cells for online monitoring wastewater in a 'real world' application.

CRediT authorship contribution statement

Minh Hang Do: Investigation, Writing - original draft, Methodology, Formal analysis, Data curation. **Huu Hao Ngo:** Supervision, Investigation, Project administration, Conceptualization, Writing - review & editing. **Wenshan Guo:** Supervision, Investigation, Writing - review & editing. **Soon Woong Chang:** Investigation, Project administration, Writing - review & editing. **Dinh Duc Nguyen:** Methodology, Formal analysis, Resources, Writing - review & editing. **Lijuan Deng:** Investigation, Methodology, Formal analysis. **Zhuo Chen:** Investigation, Writing - review & editing. **Tien Vinh Nguyen:** Methodology, Resources, Writing - review & editing.

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.

Acknowledgement

This research was supported by University of Technology Sydney, Australia (UTS, RIA NGO) and the Korea Institute of Energy Technology Evaluation and Planning (KETEP) and the Ministry of Trade, Industry & Energy (MOTIE) of the Republic of Korea (Grant No. 20173010092470).

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