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Single chamber microbial fuel cell for environmental monitoring of nitrate

Pile à combustible microbienne à chambre unique pour la surveillance environnementale des nitrates

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RÉSUMÉ

Les piles à combustible microbien (MFC) sont des dispositifs bio-électrochimiques qui produisent de l'électricité en utilisant des bactéries électroactives (EAB) comme moyens de surveillance de l'environnement. Dans cette étude, nous avons développé une MFC composée d'une chambre unique, imprimée en 3D, à circuit fermé pour la détection des nitrates dans l'eau. Les MFC ont été mûries à l'aide d'eaux usées et d'eaux usées synthétiques, ce qui a donné une tension de sortie maximale de 295 mV. Les résultats de laboratoire ont montré une sensibilité élevée du biocapteur MFC fait en tissu de carbone pour la détection du nitrate avec une détection minimale de 0,625 mg/L (des concentrations plus faibles seront testées avant la conférence). De plus, de vrais échantillons d'eau environnementale ont été testés avec le biocapteur MFC et ont montré des résultats prometteurs. Nos travaux futurs porteront sur l'augmentation de la sensibilité de la MFC en optimisant le matériel de l'anode ainsi que de la résistance externe.

ABSTRACT

Microbial fuel cells (MFC) are bio-electrochemical devices that produce electricity by employing electroactive bacteria (EAB) as environmental monitoring assets. In this study, we developed a single chamber closed-circuit 3D-printed MFC for nitrate detection in water. The MFCs were matured by wastewater and synthetic wastewater, resulting in a maximum voltage output of 295 mV. The result of laboratory tests showed high sensitivity of carbon cloth MFC biosensor towards detection of nitrate with a minimum detection of 0.625 mg/L (lower concentrations will be tested prior to the conference). Moreover, real environmental water samples were tested by the MFC and showed promising results. Our future work will increase the sensitivity of the MFC by optimising the anode electrode material and external resistance.

KEYWORDS

Air cathode, Biosensor, Microbial Fuel cell, Nitrate, Single chamber

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1 INTRODUCTION

Anthropogenic activities have resulted in increased use and discharge of nitrate into the ecosystem from point sources such as wastewater discharges and non-point sources such as agricultural fertilisers. Increased nitrate levels have induced eutrophication of water bodies causing serious health issues for human and aquatic life. Due to the low sensitivity and economic disadvantages of current lab-based and online nitrate detection methods, researchers have suggested alternative nitrate detection methods, such as biosensors (Ren et al. 2022)

Microbial fuel cells (MFC) are bio-electrochemical devices containing electroactive bacteria that consume organic carbon and produce electricity (Zhao, Slade, and Varcoe 2009). A typical MFC includes an anode and cathode electrode separated from a proton exchange membrane (PEM). The anode and cathode are placed inside an anolyte and catholyte solution; while the anode compartment works in anaerobic conditions, the catholyte is aerated with oxygen (Liu and Logan 2004). This type of MFC requires continuous catholyte aeration, which limits industrial production purposes. Single-chamber air-cathode MFCs have been introduced with limited requirements, fewer setup costs, and flexible applications, such as biosensors (Adekunle et al. 2021; Liu and Logan 2004; Wang et al. 2018).

MFC-based biosensors work with two basic principles: (a) increasing the organic carbon source for the electrochemically active bacteria (EAB) will increase the metabolic activity resulting in higher current output, and (b) increasing the disturbance for the EAB, such as pH change, toxicants, electron acceptors, temperature, and conductivity change will reduce the output current (Do et al. 2020). Electron acceptors such as nitrate in a single chamber MFC will compete with the anode electrode for the generated electrons by EAB and therefore reduce the electrical potential of the anode electrode (Ren et al. 2022; Sukkasem et al. 2008).

Sukkasem et al. (2008) investigated the effect of nitrate in a closed circuit MFC and found a positive correlation between nitrate concentration and voltage output. Ren et al. compared the performance of open and closed-circuit microbial fuel cell for monitoring the nitrate in water. The results showed higher sensitivity of open circuit biosensor towards nitrate with higher response time compared to closed circuit MFC. Although previous studies showed promising results towards the application of MFC for nitrate monitoring, these results are purely based on artificial synthetic wastewater feed with constant levels of organic matter, pH, temperature, and other constituents. Therefore, further research is required to investigate the in-situ application of MFC biosensor to monitor the nitrate level in environmental waters.

In this study, a 3D-printed single chamber air-cathode MFC for monitoring of NO_3 ions in water was constructed. The effect of various NO_3 concentrations on voltage output was studied. Further validation of the biosensor was performed using real river water samples.

2 MATERIAL AND METHODS

2.1 Construction and start-up of the MFC biosensor

The air-cathode single-chamber MFCs were designed in Solidworks (2021) and printed with a rapid prototype 3D printer (Creator 3, Flash Forge, China). 50 mL working volume of the anode chamber was separated by a proton exchange membrane (Nafion 117, Sigma-Aldrich, USA) hot pressed on a Pt-coated carbon cloth cathode electrode (Flexible carbon cloth + 0.5 mg/cm² Pt, FuelCellEarth, USA). This study used plain carbon cloth as anode electrodes (FuelCellEarth, USA). Copper wires connected the electrodes with a 1000 Ω external resistor. An Arduino-based BOSL board (www.bosl.com.au) developed at Monash University was used to record the voltage difference between anode and cathode electrodes and record the data on an SD card every minute.

To provide the anode electrode with a suitable range of bacteria, the MFCs were matured by injecting wastewater from the primary clarifier of a sewage treatment plant in Melbourne, Australia. An acetate medium (1000 mg/L) was used as synthetic wastewater that contained 50 mM phosphate buffer solution and minerals, as reported by Liu and Logan (2004). Each MFC was injected with pure wastewater for three days, and then a mixture (1:1, V/V) of the raw wastewater and synthetic wastewater until a stable voltage was recorded for 24 hours. Then, the MFCs were only fed with synthetic wastewater. Nitrogen gas was purged into all solutions for 10 minutes before injection to remove the oxygen. All the experiments took place at room temperature of 23 ± 2 °C.

2.2 Nitrate tests of the MFC biosensor

After maturing, the MFC biosensors were first tested using synthetic wastewater mixtures containing various concentrations of sodium nitrate. After each test, fresh synthetic wastewater without any nitrate was injected into the MFC for recovery. To validate the findings of the synthetic wastewater mixtures, environmental waters were collected and tested. Each environmental water sample was split into: (1) a 25 mL aliquot was filtered with 50 μ m filter paper and analysed for NOx, and nitrate concentration using FIA and (2) a 50 mL aliquot was deoxygenated using N₂ gas for 10 minutes and then injected into the carbon cloth MFC biosensors with no further modification (and no pre-filtering to represent direct field usage).

3 RESULTS AND DISCUSSION

3.1 Nitrate tests of MFC biosensor

A reduction in the voltage output after introducing the synthetic wastewater mixtures with various concentrations of nitrate to the MFC was observed in Figure 1 (a). The NO $_3$ ions are common electron acceptors of microbial metabolism and compete with the anode electrode (Yi et al., 2020). Also, the resting voltage is related to the NO $_3$ concentration in the solution; when exposed to a high level of NO $_3$, the minimum stable voltage is higher than when the sensor is exposed to a lower level of NO $_3$. Indeed, Figure 1 (b) shows the corresponding correlation between ΔV and the nitrate concentrations. The logarithmic line of best fit was chosen as per previous research on nitrate MFC biosensors (Ren et al. 2022).

In Figure 1 (c), the corresponding ΔV for the environmental samples was much higher than the laboratory results. This can be explained by the lower concentration of organic carbon compared to the laboratory samples (1000 mg/L NaAc). However, as can be seen by increasing the nitrate concentration, ΔV of the MFC biosensor was decreased, which is the opposite of synthetic wastewater results. Since the PEM was not pre-treated, which caused biofouling of Nafion after 5 months of operation, the nitrate reduction process on the cathode electrode acted as the alternative electron acceptor. Therefore, by increasing the nitrate concentration, an increase in the output voltage was observed. These results align with a study by Li et al., (2014) which used metals as electron acceptors in an oxygen-limited MFC. Using the correlation presented in Figure 1 (d), the nitrate concentration of an environmental water sample was predicted to be 3.6 mg/L, while the actual nitrate concentration of the sample was analysed to be 4.1 mg/L. The model prediction accuracy is more than 87% based on these results.

The lowest nitrate detection range with a closed circuit MFC has been recorded as 1 mg/L (Ren et al. 2022), while the MFC of this study was able to detect nitrate as low as 0.625 mg/L with the same conditions. However, the response time for this MFC biosensor is 40 minutes, which is not practical for field deployment purposes. The long response time is hypothesised to be the biofouling of the not pretreated Nafion. Moreover, another prototype of MFC with carbon felt anode electrode and pre-treated PEM was constructed in this study. Carbon felt MFC biosensor's response time was much shorter (almost 10 minutes). The low response time and recovery time of the carbon felt MFC biosensor shows great potential for online monitoring of the toxicants and, therefore, worth more investigations.

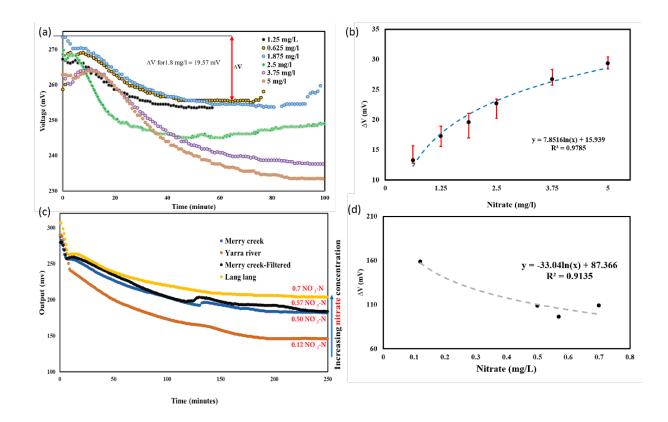


Figure 1: (a) Output voltage results of the MFC biosensor injected with a mixture of synthetic wastewater with various concentrations of NO_3^- , (b) the corresponding correlation between NO_3^- concentration and ΔV , (c) output voltage results of carbon cloth MFC biosensor injected with environmental samples, (d) corresponding correlation with nitrate concentration and output voltage. The error bars show the experiment replicates.

4 **CONCLUSION**

A 3D-printed single-chamber air-cathode MFCs was produced as nitrate biosensors in water. The PEM of the carbon cloth MFC was not pre-treated and, as a result, generated lower output voltage and higher response time compared to the MFC with pre-treated PEM. The results showed a high sensitivity for nitrate MFC with the lowest detection range of 0.6 mg/L. The corresponding correlation of ΔV and nitrate concentration resulted in an R-squared coefficient of R^2 = 0.97. This is the first study investigating nitrate's effect in environmental water using an MFC biosensor. The environmental samples' results showed that a higher nitrate concentration generates a higher voltage output. These results confirmed that the nitrate reduction process was used as the electron acceptor on the cathode electrode. Moreover, the nitrate concentration of the real environmental water sample (overall 4.1 mg/L nitrates) was predicted to be 3.6 mg/L, which shows the significant accuracy of the model.

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