

© <2021>. This manuscript version is made available under the CC-BY-NC-ND 4.0 license  
<http://creativecommons.org/licenses/by-nc-nd/4.0/>  
The definitive publisher version is available online at <https://doi.org/10.1016/j.scitotenv.2021.148755>

**Performance of a dual-chamber microbial fuel cell as biosensor for on-line measuring ammonium nitrogen in synthetic municipal wastewater**

Minh Hang Do<sup>1</sup>, Huu Hao Ngo<sup>1,2,8,\*</sup>, Wenshan Guo<sup>1,7</sup>, Soon Woong Chang<sup>3</sup>, Dinh Duc Nguyen<sup>3,4</sup>, Pooja Sharma<sup>5</sup>, Ashok Pandey<sup>5,6</sup>, Xuan Thanh Bui<sup>7</sup>, Xinbo Zhang<sup>8</sup>

<sup>1</sup>*Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NWS 2007, Australia*

<sup>2</sup>*NTT Institute of Hi-Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam*

<sup>3</sup>*Department of Environmental Energy Engineering, Kyonggi University, 442-760, Republic of Korea*

<sup>4</sup>*Institution of Research and Development, Duy Tan University, Da Nang, Vietnam*

<sup>5</sup>*Center for Energy and Environmental Sustainability, Lucknow 226 022, Uttar Pradesh, India*

<sup>6</sup>*Centre for Innovation and Translational Research, CSIR-Indian Institute of Toxicology Research, Lucknow-226 001, India*

<sup>7</sup>*Faculty of Environment and Natural Resources, Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City 700000, Viet Nam*

<sup>8</sup>*Joint Research Centre for Protective Infrastructure Technology and Environmental Green Bioprocess, Department of Environmental and Municipal Engineering, Tianjin Chengjian University, Tianjin 300384, China*

\*Corresponding author: Email: ngohuuhaol21@gmail.com

## Abstract

This study investigates the performance of microbial fuel cells (MFC) for on-line monitoring ammonium ( $\text{NH}_4^+\text{-N}$ ) in municipal wastewater. A double chamber microbial fuel cell (MFC) was established in a continuous mode under different influent ammonium concentrations ranging from 5 to 40  $\text{mg L}^{-1}$ . Results indicated that excess ammonium would inhibit the activity of electrogenic bacteria in the anode chamber and consequently affect electricity production. An inversely linear relationship between concentration and voltage generation was obtained with coefficient  $R^2$  0.99 and the MFC could detect up to 40  $\text{mg L}^{-1}$  of  $\text{NH}_4^+\text{-N}$ . Notably, no further decline was observed in voltage output and there was in fact a further increase in ammonia concentration ( $> 40 \text{ mg L}^{-1}$ ). The stability and high accuracy of ammonium-based MFC biosensors exposed competitive results compared to traditional analytical tools, confirming the biosensor's reliability. Furthermore, pH 7.0;  $R$  1000  $\Omega$  and HRT of 24 h are the best possible conditions for the MFC biosensor for monitoring ammonium. The simplicity in design and operation makes the biosensor more realistic for practical application.

**Keywords** Microbial fuel cell, ammonium, municipal wastewater, biosensor, stability, voltage output

## 1.Introduction

There are various methods and techniques for measuring ammonium nitrogen, in which spectrophotometric, fluorometric, ion-selective electrode method, and electrochemical are the most commonly used in the aquatic ecosystem. Spectrophotometric methods based on Nessler's reagent method, Indophenol blue method, and gas diffusion are considered the prevalent tool for analyzing because simple in use, no heating required. However, the reagent used in this method is toxic and causes some effects on health and environmental issues (Crespo, 2017; Lin et al., 2019).

Fluorometric methods involve the reaction between ammonium with o-phthaldialdehyde (OPA) reaction, which is suitable for trace ammonia nitrogen measurement due to their high

sensitivity in detection without enrichment techniques (Zhu et al., 2019). However, there are two significant issues in the trace analysis, including methodologies for storing samples and blank samples, resulting in contamination and requiring attention during the analysis.

Electrochemical methods including conductometric, voltammetric, potentiometric, and amperometric have been utilized to analyze ammonium with fast response time, fewer reagents needed and low cost (Lin et al., 2019). The ion-selective electrode method (ISE) is suitable for in-situ analysis with the advantages of easy operation, low cost in manufacturing, and fast response (Zhu et al., 2019). Nevertheless, a significant disadvantage in the ISE method is the effect of interfering ions in the water samples to be analyzed (Dineski et al., 2010) such as low resolution, low accuracy, and limited shelf life (Pellegrin et al., 2016), inappropriate for application in trace ammonium concentration analysis, and a long equilibration time of direct analysis (Cao et al., 2016).

These traditional  $\text{NH}_4^+\text{-N}$  analytical methods are usually time-consuming and unsuitable for online monitoring due to the large size and complexity of the instruments. Thus, developing a fast, sensitive, and cost-effective analytical method for online monitoring ammonium nitrogen is necessary.

Microbial fuel cells (MFCs) are increasingly regarded as an innovative technology for treating wastewater and generating electricity at the same time (Chouler and Lorenzo, 2015; Do et al., 2020a). MFCs have been employed to act as a biosensor for monitoring water quality such as BOD, nitrate, toxicity, pathogenic bacteria and volatility fatty acids (VFA) (Do et al., 2020b). MFC biosensors can be operating without any further transduction signal device, so this technology is expected to be a rapid and real-time monitoring of water quality. However, to date, current knowledge of monitoring ammonium in wastewater is still limited. Therefore, discovering a simple, rapid and accurate response for on-line measuring ammonium is necessary.

Generally, a microbial fuel cell is a bio-electrochemical device that can convert chemical energy directly to electrical energy based on electrochemically bacterial catalytic activity for

oxidation of the organic compound (Logan et al., 2006). MFC's basic schematic includes two compartments, an anaerobic anode and aerobic cathode separated by a proton exchange membrane (PEM). The anode chamber plays a vital role in the detection phase. Electrons and protons are the products of the degrading organic substrate process via the microorganism's metabolic process. Electrons then migrate to the cathode chamber via external resistance and combine with an electron acceptor (e.g.,  $O_2$ ,  $KMnO_4$ , etc.) to complete the oxygen reduction reaction (ORR) (Chaturvedi and Verma, 2016). Electricity can be generated from various biodegradable organic materials in MFC, including carbohydrates, low molecular weight organic acids, protein, waste, and waste streams such as dairy manure, domestic wastewater, and food process wastewater. Literature research has indicated that the MFC's performance is influenced by various operating and design parameters such as pH, electrical conductivity, flow rate, substrate types, and electrode materials (Kim et al., 2007; Mardanpour et al., 2017; Oh and Logan, 2006). Di Lorenzo et al. (2014) illustrated that anodic bacterial activities directly affect the MFC's electrical current generation. Any changes in the operating elements or environmental conditions such as organic or nutrient loading rate caused an effect on the electrons transport mechanism to the anode, consequently generating a measurable change in the output current (Di Lorenzo et al., 2009, Stein et al., 2010). Therefore, MFCs can be employed as a valuable device for monitoring wastewater quality.

Hussain et al. (2016) revealed energy creation capability in closed-circuit MFC when ammonium was the sole substrate in an anolyte solution. In this research, the voltage output of  $225 \pm 6$  mV was observed at  $200 \text{ mg L}^{-1}$  ammonium concentration. This result indicated ammonium's potential to serve as an electron donor for electricity generation by direct oxidation at the anode. Ammonium may contribute to electricity generation in MFC in two ways. The first way is that ammonium can supply electrons via its oxidation under both aerobic and anaerobic conditions, which exhibit negative Gibbs free energy ( $\Delta G$ ) (Kumar et al., 2017). Consequently, the standard potential for both reaction is positive, illustrating the capability to produce electricity in MFC

with ammonium as an electron donor (anode). The second way is that ammonium in the anode chamber may be used by nitrifying bacteria to produce organic compounds that are used by heterotrophs to generate electricity (Kindaichi et al., 2004).

This study is the first investigation on the performance of a double chamber microbial fuel cell at a continuous mode for on-line monitoring ammonium in municipal wastewater. The effects of the anodic pH, fuel feeding rate and the catholyte solution on the MFC biosensor were investigated while the relationship between ammonium concentration with a voltage output of biosensor, reaction time and its stability, and MFC biosensor cross-validation with analytical methods were evaluation.

## 2. Materials and methods

### 2.1. The microbial fuel cell system

A double-chamber microbial fuel cell in a rectangular shape was designed for this study according to previously studied (Do et al., 2020a). The practical volumes of the anode and cathode compartment are 300 and 400 mL, respectively. There are two holes on each side of the reactor for influent and effluent flow. The other two holes are set up on the top of the anode reactor, one for the anode electrode and one for the reference electrode. Proton exchange membrane PEM (Nafion 5 cm in diameter) was utilized to separate the two compartments. A carbon felt 3 cm in diameter and 0.6 cm in thickness was used as an anode electrode, while a carbon-fiber brush 3.0 cm in diameter and 2.5 cm in brush length was used in the cathode chamber. External resistance  $R=1000 \Omega$  connected the two electrodes via a copper wire. The anode reactor was kept in an anaerobic state while the cathode was open to the air. The configuration and the function of dual chamber MFC system as a biosensor are shown in Figures 1a and 1b.

**Fig. 1:**

### 2.2. Synthetic wastewater

Synthetic wastewater was prepared with the following components:  $\text{NH}_4\text{Cl}$ ,  $5.4 \text{ mg dm}^{-3}$ ;  $\text{KH}_2\text{PO}_4$ ,  $20 \text{ mg dm}^{-3}$ ;  $\text{MgSO}_4 \cdot 7 \text{ H}_2\text{O}$ ,  $0.4 \text{ mg dm}^{-3}$ ;  $\text{CaCl}_2 \cdot 2 \text{ H}_2\text{O}$ ,  $32 \text{ mg dm}^{-3}$  yeast and  $0.61 \text{ ml}$  of trace nutrients. Glucose was utilized as the carbon source. The trace nutrient solution include  $0.275 \text{ mg L}^{-1} \text{MnCl}_2 \cdot 7\text{H}_2\text{O}$ ,  $0.44 \text{ mg L}^{-1} \text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $1.45 \text{ mg L}^{-1} \text{FeCl}_3$ ,  $0.391 \text{ mg L}^{-1} \text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $0.42 \text{ mg L}^{-1} \text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ . The solution was plugged with nitrogen gas for 30 minutes before feeding to ensure the anaerobic condition of the anode chamber.

The operating temperature of the biosensor system was kept at room temperature ranging from  $25\text{-}33 \text{ }^\circ\text{C}$ . The ammonium concentration was adjusted by utilizing  $\text{NH}_4\text{Cl}$ . All the chemicals were purchased from Sigma Aldrich.

### 2.3 Enrichment process

$100 \text{ mL}$  anaerobic sludge (from Cronulla wastewater treatment plant) and  $200 \text{ mL}$  artificial wastewater ( $\text{COD } 300 \text{ mg L}^{-1}$ ) were added to the anode chamber to inoculate and enrich the microbial activity. This process was conducted under a closed circuit with  $R \text{ } 1000 \text{ } \Omega$  and was carried out until the observed stable voltage output. Then MFC biosensor was performed in a continuous mode with municipal wastewater and no inoculum added.

### 2.4 MFC operation

Artificial wastewater was added from the  $1000 \text{ ml}$  glass bottle into the anode chamber by utilizing a peristaltic pump. The MFC was operated continuously under the stable COD

concentration of 300 mg L<sup>-1</sup> at room temperature (21-25 °C). The anolyte's pH was controlled by NaOH or HCl. The cathode reactor was plugged with air to provide oxygen for the cathode reaction. The catholyte pH was maintained in a neutral state during the whole experiment using phosphate buffer saline (PBS). The relationship between the MFC's voltage output with the ammonium concentration was established in this experiment with six levels, these being 5, 10, 15, 20, 30 and 40 mg L<sup>-1</sup>. Also, the influence of different pH, the fuel feeding rate and catholyte solution on NH<sub>4</sub><sup>+</sup>-N detection and MFC's energy production were investigated.

### 2.5 Calculation

The anode chamber's input and the output sample were taken and analyzed daily, including COD, NH<sub>4</sub><sup>+</sup>-N. COD concentration was tested following standard methods (APHA, 1998). NH<sub>4</sub><sup>+</sup>- concentrations were determined through the spectrophotometric method utilizing Spectroquant test kit (NOVA 60, Merck). The voltage generated between electrodes was measured continuously every 5 minutes by a voltage data logger (Madge Tech 101A) directly connected to the computer. The sample's pH was measured by a pH meter (HI9025, Hanna Instruments, Limena, Italy).

The current (I) is calculated according to Ohm law (Carter, 2013).

$$I=U/R \text{ (mA)} \quad (1)$$

The power output (P) and current density (*i*) are calculated using the following equations:

$$P= U*I \quad \text{(mW)} \quad (2)$$

$$i=I/A \quad \text{(mA/m}^2\text{)} \quad (3)$$

*U* (mV): Voltage measured between anode and cathode

*R* (Ω): external resistance

*A*: Anode electrode's surface area (cm<sup>2</sup>)

## 3 Results and discussion

### 3.1 Effect of the anodic pH



pH is one of the most vital operating parameters that directly affect microorganisms' activity in the anode compartment and protons transport mechanism (Jia et al., 2014; Yuan et al., 2011). In this study, various pH ranges from 5 to 9 were tested under the biosensor's voltage and power output function. The MFC biosensor was operating under COD 300 mg L<sup>-1</sup>, fixed external resistant R 1000 Ω, and the catholyte solution's pH was kept stable at neutral pH.

As shown in Figure 2, the highest voltage output and power density of the MFC biosensor were obtained between pH 7 and 8. The maximum voltage was 456.5, 452.5 mV at pH 7, 8 respectively. A minor change in the voltage output can be found when increasing pH up to 9, however, there was about 23.23% reduction in voltage output at pH 5 compared to that at pH 7. A similar trend was discovered with the power density of the MFC biosensor.

**Fig 2:**

It can be illustrated that neutral pH is the optimal value of the MFC biosensor's electricity generation. The reason might be that the activity of electrogenic bacteria was inhibited in the acidification environment, affecting the biofilm formation of the anode surface and the stability of the MFC biosensor (Behera et al., 2010; Patil et al., 2011; Yuan et al., 2011). This present result is consistent with previous research by (Zhang et al., 2011; Zhuang et al., 2010). Previous studies indicated the influence of pH on the development of the anode biofilm (Oliveira et al., 2013; Zhang et al., 2011). Their results also revealed that the thickness and the diversity of microbes were less at the neutral condition in the acidic environment.

### **3.2. Effect of fuel feeding rate**

This study was conducted with constant influent COD of 300 mg L<sup>-1</sup>. The effect of different anolyte flow rates ranging from 0.25, 0.35, 0.5, and 0.7 mL min<sup>-1</sup> resulting in the flowing HRT 24h, 16.67h, 11.67h, and 8.33h was evaluated. The MFC performance was established as a function of electric generation, COD removal efficiency. During the experiment, the

concentration of the catholyte was kept stable at 100 mM NaCl+50mM PBS. Each period of the experiment lasted two weeks. The voltage output was measured continuously by the voltage data logger every 5 minutes.

Overall, the voltage output production in MFCs was inversely proportional to the anolyte flow rate during this experiment. As can be seen in Figure 3, the maximum voltage output of the MFC was 435.5 mV at the fuel rate 0.25 and its value declined to 375.6 mV when raising the anolyte flow rate to 0.7 mL min<sup>-1</sup>. This might be because a higher flow rate of feed solution could not benefit the anode compartment's mixing conditions. The depletion of anaerobic microbes in the anode compartment might cause some harm to the process of energy creation (Sobieszuk et al., 2017). These above reasons might significantly influence the concentration and growth of electricity bacteria, decreasing the electricity generation of MFCs. Moreover, anolyte's higher flow rate leads to a higher OLR, which caused some blockage of pores on the anode electrode's surface. Consequently, preventing the transport of the electrochemically bacterial and current to the electrode surface reduced the current generation in MFC (Ma et al., 2016). In another research by Elakkia and Matheswaran. (2013), the authors also illustrated that higher OLR caused a higher risk for membrane fouling, which led to a worse influence on MFC's power generation. Furthermore, excessive organics could facilitate the activity of methanogenic microorganisms, reducing the electrogenic bacteria's capacity. Therefore, the fuel feeding rate of 0.25 mL min<sup>-1</sup> was applied as the optimal value in operating MFC biosensor.

In MFC, the COD reduction efficiency was executed by degrading the organic matter by microbial species community and concentration in the anode compartment. The COD removal rate was effective with the different anolyte flow rates, which vary from the MFC, which decreased as the anolyte flow rate rose from 0.25 to 0.7 mL min<sup>-1</sup>. Around 79.5 to 85% of the COD was removed at the flow rate range of 0.25 to 0.5 mL min<sup>-1</sup>. However, its value decreased to 70% as the flow rate increased to 0.7 mL min<sup>-1</sup>. In contrast, the removal efficiency of ammonium in the anode chamber varied only in a small way at different anolyte flow rates.

These results illustrated that ammonium removal efficiency is not significantly affected by the difference in anolyte flow rate.

**Fig 3:**

### ***3.3 Effect of the catholyte solution***

The catholyte solution's impact on the current production was investigated under the different concentrations of the NaCl and phosphate buffer saline (PBS). COD concentration was maintained at  $300 \text{ mg L}^{-1}$ ,  $R 1000 \Omega$ , airflow rate at the cathode was  $100 \text{ mL min}^{-1}$  during the experiment process. The data shows that the highest power density was obtained with 100 mM NaCl and 50 mM PBS, followed by 50 mM NaCl and deionized water only (Figure 4). Therefore, 100 mM PBS and 50mM NaCl was the best component of the catholyte solution for electricity production in this study.

The reason is that the catholyte solution's ionic strength has an impact on the energy production of the MFCs. This present result is consistent with previous researches. For example, Huang et al. (2010) concluded that increased electrolyte ionic strength could reduce MFC's internal resistance and improve the power output. Furthermore, Nam et al. (2010) has revealed an increase in the power output of MFCs with a rise of PBS concentration in a particular range.

**Fig 4:**

### ***3.4. Relationship between ammonium concentration with a voltage output of biosensor***

Under optimal operating conditions, the correlation between ammonium concentration and the biosensor's voltage output was investigated. Ammonium chloride ( $\text{NH}_4\text{Cl}$ ) was used for maintaining respective ammonium concentrations in the feed. Each period of the experiment lasted two weeks. NaCl 100 mM+50mM PBS was utilized as catholyte with airflow speed of  $100 \text{ mL min}^{-1}$  during the whole experiment.

**Table 1:**

As shown in Figure 5a, the maximum voltage output value was 435.5 mV at 5 mg L<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N. Noticeably, there was a decrease in the MFC's electricity creation as the influent NH<sub>4</sub><sup>+</sup>-N concentration rising from 5 to 40 mg L<sup>-1</sup>. The possible reason is that higher ammonia inhibits the activities of exoelectrogens in MFC, consequently affecting MFC's electricity production. Hansen et al.(1998) and Müller et al. (2006) reported that ammonium nitrogen has a negative impact on the microbial community. The toxicity of ammonia has been characterized by two mechanisms: (i): the cytosolic enzyme activity was inhibited by un-ionized NH<sub>3</sub>; (ii) the accumulation in the cell of NH<sub>4</sub><sup>+</sup> from hydrophobic NH<sub>3</sub> caused an inhibition by changing the intracellular pH conditions. Moreover, (de Baere et al., 1984) also indicated that a high concentration of ammonia salt might cause the dehydration of bacteria due to osmotic stress.

Furthermore, Nam et al. (2010b) illustrated that ammonium might act as a building block for the microorganism's transport mechanism to the anode electrode. Figure 5b expose the highest current density is 173.4 mA m<sup>-2</sup> at 5 mg L<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N. The current density decreased by 30% when the influent ammonium concentration increased eight-fold to 40 mg L<sup>-1</sup>.

The inhibition of ammonia on the power generation in MFC has been demonstrated in previous researches (Hügemann et al., 2016; Kim et al., 2011; Müller et al., 2006; Rittmann and McCarty, 2001; Tice and Kim, 2014). However, a different conclusion about the level of ammonia concentration limits MFC's energy production in these previous studies. For example, Nam et al. (2010b) illustrated that the current generation in a single MFC was inhibited while rising the concentration of NH<sub>4</sub><sup>+</sup>-N excess 500 mg L<sup>-1</sup>. In another study by Kim et al. (2011), a further increase in total ammonia nitrogen upper 3500 mg N L<sup>-1</sup> leads to significant inhibition of MFC's maximum power density. However, some other researchers also reported that the MFC's electricity production was still high at the total ammonia concentration of 4000 mg N L<sup>-1</sup> (Tice

and Kim, 2014). The varying results of the ammonium thresholds may be attributed to various substrates used, operating parameters, biological factors, and the MFC configuration system.

**Fig 5:**

An adverse correlation ship between ammonium concentration up to 40 mg L<sup>-1</sup> and the maximum voltage output was shown in Figure 6. A slope of 3.15 and a coefficient (R<sup>2</sup>) of 0.9071 was found. These results demonstrated that the MFC's voltage was directly related to the influent feeding NH<sub>4</sub><sup>+</sup>-N concentration. This correlation can potentially be used toward the development of a biosensor for real-time monitoring of NH<sub>4</sub><sup>+</sup> N.

As reported, electricity production in MFCs depends on electrochemical bacteria's activity that forms a biofilm on the anode. In the previous research by (Hussain et al., 2016), the authors revealed energy creation capability in closed circuit MFC when ammonium was the sole substrate in anolyte solution. In this research, the voltage output of 225±6 mV was observed at 200 mg L<sup>-1</sup> ammonium concentration. This result indicated ammonium's potential to serve as an electron donor for electricity generation by direct oxidation at the anode.

**Fig 6:**

### 3.5 Reaction time

The reaction time of the MFC-based biosensor for monitoring ammonium concentration is the critical indicator that impacts the accuracy of the measured signal. Longer reaction time is not suitable for commercial applications, however, the reaction time needs to be long enough for completing the biochemical reaction in the biosensor and give the accuracy signal.

In this research, the sensor signal at the steady-state was obtained at approximately 1.2 hours. Besides, it was noted that the higher ammonium concentration, the longer was the reaction time. In general, a biosensor's reaction time was varied and lasted 5 minutes to 10 hours or some days, depending on the types of used substrate, inoculum, and operating conditions of the MFC.

### **3.6 Stability of the biosensor**

Stability is regarded as a requirement of a reliable biosensor. A biosensor's stability mainly focuses on the configuration, immobilization methods, and substrates used. The ammonium MFC biosensor's stability was investigated with a fixed ammonium concentration of  $5 \text{ mg L}^{-1}$  for one month with other optimal operation conditions. The signal output (voltage) at the stable stage was measured. The average voltage output of the biosensor was 428.35 mV with a standard deviation of  $\pm 3.98\%$ . This current result of the study indicates that MFC can be utilized as a reliable biosensor for monitoring ammonium nitrogen. All data of this experiment was collected from the voltage data logger during 30 days (Figure 7).

**Fig 7:**

### **3.7 MFC biosensor cross-validation with analytical methods**

The ammonium concentration was determined by a spectrophotometric method utilizing a Spectroquant test kit (NOVA 60, Merck). Comparison of ammonium detection in wastewater by MFC biosensor with conventional methods was illustrated in the table below with a relative error from 0.39 to 2.7%. This variation in the biosensor's measured signal was acceptable and it ensured the success of MFC biosensor for monitoring ammonium concentration in wastewater.

**Table 2:**

### **3.8 Morphology of the attached biofilm on the anode electrode**

Yang et al.(2012) illustrated that the matured biofilm in the anode surface significantly influences the electron mechanism from the microbes to the electrode. The morphology of the attached biofilm on the anode electrode was conducted by scanning electron microscope (Zeiss Supra 55VP, Carl Zeiss AG). Firstly, one small piece of carbon felt in the anode electrode was cut in 1x1cm, then directly fixed in 2.5% glutaraldehyde with 0.1M PBS in 4 hours. Secondly, the sample was dehydrated with dilute ethanol solutions ranging from 30 to 100% and continued

dried with a tert-butylethanol solution. Lastly, a thin gold layer was covered on the sample and be analyzed in a high vacuum condition.

Figure 8a depicts the pure carbon felt structure, including long straight carbon fiber lying across each other without microorganisms present. Closed-up images (Figures 8b,8c,8d) illustrated that the significant thick and dense biofilm were developed and covered with large colonies of bacteria morphology, in which a rod-type species was predominant.

**Fig. 8:**

#### **4 Conclusion**

For the first time, the present work demonstrated the feasibility of the MFC-based biosensor for ammonium nitrogen monitoring in municipal wastewater. Results shows the negative impact of excessive ammonium nitrogen on the electricity production of the MFC biosensor. A relation between voltage output and ammonium concentration was inversely linear with a high coefficient  $R^2$ . The operating conditions such as fuel feeding rate and catholyte solution on the biosensor system were successfully investigated and convinced that the ammonium could be detected by dual-chamber MFC and generated electricity simultaneously. The stability stage of the biosensor could be reached in the response time of approximately 1.2 hours. The precision of the biosensor for ammonium measurement is comparative with the traditional method, and the results indicate the reliability of the biosensor. Overall, the key findings of this research help to promote the practical application of MFC biosensor for on-line monitoring ammonium nitrogen.

#### **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), Republic of Korea (No. 20183020141270 and No. 20194110300040).

Journal Pre-proof



## References

- Behera, M., Jana, P.S., More, T.T., Ghangrekar, M.M. 2010. Rice mill wastewater treatment in microbial fuel cells fabricated using proton exchange membrane and earthen pot at different pH. *Bioelectrochemistry*, **79**(2), 228-233.
- Chaturvedi, V., Verma, P. 2016. Microbial fuel cell: a green approach for the utilization of waste for the generation of bioelectricity. *Bioresources and Bioprocessing*, **3**(1), 38.
- Chouler, J., Lorenzo, M.D. 2015. Water Quality Monitoring in Developing Countries; Can Microbial Fuel Cells be the Answer? *Biosensors*, **5**(3), 450-470.
- Di Lorenzo, M., Thomson, A.R., Schneider, K., Cameron, P.J., Ieropoulos, I. 2014. A small-scale air-cathode microbial fuel cell for on-line monitoring of water quality. *Biosensors and Bioelectronics*, **62**, 182-188.
- Do, M.H., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Deng, L., Chen, Z., Nguyen, T.V. 2020a. Performance of mediator-less double chamber microbial fuel cell-based biosensor for measuring biological chemical oxygen. *Journal of Environmental Management*, **276**, 111279.
- Do, M.H., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Varjani, S., Kumar, M. 2020b. Microbial fuel cell-based biosensor for online monitoring wastewater quality: A critical review. *Science of The Total Environment*, **712**, 135612.
- Elakkiya, E., Matheswaran, M. 2013. Comparison of anodic metabolisms in bioelectricity production during treatment of dairy wastewater in Microbial Fuel Cell. *Bioresource Technology*, **136**, 407-412.
- Gonzalez del Campo, A., Lobato, J., Cañizares, P., Rodrigo, M.A., Fernandez Morales, F.J. 2013. Short-term effects of temperature and COD in a microbial fuel cell. *Applied Energy*, **101**, 213-217.

- Hasan, H.A., Abdullah, S.R.S., Kamarudin, S.K., Kofli, N.T. 2011. Response surface methodology for optimization of simultaneous COD, NH<sub>4</sub><sup>+</sup>-N and Mn<sup>2+</sup> removal from drinking water by biological aerated filter. *Desalination*, **275**(1), 50-61.
- Hiegemann, H., Herzer, D., Nettmann, E., Lübken, M., Schulte, P., Schmelz, K.-G., Gredigk-Hoffmann, S., Wichern, M. 2016. An integrated 45L pilot microbial fuel cell system at a full-scale wastewater treatment plant. *Bioresource Technology*, **218**, 115-122.
- Hobbie, S.E., Finlay, J.C., Janke, B.D., Nidzgorski, D.A., Millet, D.B., Baker, L.A. 2017. Contrasting nitrogen and phosphorus budgets in urban watersheds and implications for managing urban water pollution. *Proceedings of the National Academy of Sciences*, **114**(16), 4177-4182.
- Huang, J., Sun, B., Zhang, X. 2010. Electricity generation at high ionic strength in microbial fuel cell by a newly isolated *Shewanella parvisflavi* EP1. *Applied Microbiology and Biotechnology*, **85**(4), 1141-1149.
- Hussain, A., Manuel, M., Tartakovsky, B. 2016. A comparison of simultaneous organic carbon and nitrogen removal in microbial fuel cells and microbial electrolysis cells. *Journal of Environmental Management*, **173**, 23-33.
- Jia, Q., Wei, L., Han, H., Shen, J. 2014. Factors that influence the performance of two-chamber microbial fuel cell. *International Journal of Hydrogen Energy*, **39**(25), 13687-13693.
- Jiang, J., Zhao, Q., Zhang, J., Zhang, G., Lee, D.-J. 2009. Electricity generation from biotreatment of sewage sludge with microbial fuel cell. *Bioresource Technology*, **100**(23), 5808-5812.
- Kim, H.-W., Nam, J.-Y., Shin, H.-S. 2011. Ammonia inhibition and microbial adaptation in continuous single-chamber microbial fuel cells. *Journal of Power Sources*, **196**(15), 6210-6213.

- Kim, J., Kim, H., Kim, B., Yu, J. 2014. Computational fluid dynamics analysis in microbial fuel cells with different anode configurations. *Water Science and Technology*, **69**(7), 1447-1452.
- Kim, J.R., Cheng, S., Oh, S.-E., Logan, B.E. 2007. Power Generation Using Different Cation, Anion, and Ultrafiltration Membranes in Microbial Fuel Cells. *Environmental Science & Technology*, **41**(3), 1004-1009.
- Kuntke, P., Śmiech, K.M., Bruning, H., Zeeman, G., Saakes, M., Sleutels, T.H.J.A., Hamelers, H.V.M., Buisman, C.J.N. 2012. Ammonium recovery and energy production from urine by a microbial fuel cell. *Water Research*, **46**(8), 2627-2636.
- Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K. 2006. Microbial Fuel Cells: Methodology and Technology. *Environmental Science & Technology*, **40**(17), 5181-5192.
- Ma, D., Jiang, Z.-H., Lay, C.-H., Zhou, D. 2010. Electricity generation from swine wastewater in microbial fuel cell: Hydraulic reaction time effect. *International Journal of Hydrogen Energy*, **41**(46), 21820-21826.
- Mangadze, T., Dalu, T., William Froheman, P. 2019. Biological monitoring in southern Africa: A review of the current status, challenges and future prospects. *Science of The Total Environment*, **646**, 1492-1499.
- Mardanpour, M.M., Yaghtmaei, S., Kalantar, M. 2017. Modeling of microfluidic microbial fuel cells using quantitative bacterial transport parameters. *Journal of Power Sources*, **342**, 1017-1031.
- Matassa, S., Batstone, D.J., Hülsen, T., Schnoor, J., Verstraete, W. 2015. Can Direct Conversion of Used Nitrogen to New Feed and Protein Help Feed the World? *Environmental Science & Technology*, **49**(9), 5247-5254.
- Müller, T., Walter, B., Wirtz, A., Burkovski, A. 2006. Ammonium Toxicity in Bacteria. *Current Microbiology*, **52**(5), 400-406.

- Nam, J.-Y., Kim, H.-W., Lim, K.-H., Shin, H.-S., Logan, B.E. 2010. Variation of power generation at different buffer types and conductivities in single chamber microbial fuel cells. *Biosensors and Bioelectronics*, **25**(5), 1155-1159.
- Oh, S.-E., Logan, B.E. 2006. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. *Applied Microbiology and Biotechnology*, **70**(2), 162-169.
- Patil, S.A., Harnisch, F., Koch, C., Hübschmann, T., Fetzer, I., Carmona-Martínez, A.A., Müller, S., Schröder, U. 2011. Electroactive mixed culture derived biofilms in microbial bioelectrochemical systems: The role of pH on biofilm formation, performance and composition. *Bioresource Technology*, **102**(20), 6633-6690.
- Rat-Valdambrini, M., Belkacemi, K., Hamoudi, S. 2012. Removal of ammonium cations from aqueous solution using arene-sulphonic acid functionalised SBA-15 as adsorbent. *Canadian Journal of Chemical Engineering*, **90**(1), 18-25.
- Rittmann, B.E., McCarty, P.L. 2001. *Environmental biotechnology: principles and applications*. McGraw-Hill Education.
- Sobieszuk, P., Zamojska-Jaroszewicz, A., Makowski, Ł. 2017. Influence of the operational parameters on bioelectricity generation in continuous microbial fuel cell, experimental and computational fluid dynamics modelling. *Journal of Power Sources*, **371**, 178-187.
- Tice, R.C., Kim, Y. 2014. Influence of substrate concentration and feed frequency on ammonia inhibition in microbial fuel cells. *Journal of Power Sources*, **271**, 360-365.
- Wang, X.L., Wu, C., Zhang, J.Q., Chi, Q.L., Tian, S.S. 2011. Acclimation Stage on the Performance of Microbial Fuel Cells Subjected to Variation in COD, Temperature, and Electron Acceptor. *Advanced Materials Research*, **183-185**, 2346-2350.
- Yang, S., Du, F., Liu, H. 2012. Characterization of mixed-culture biofilms established in microbial fuel cells. *Biomass and Bioenergy*, **46**, 531-537.

- Ye, Y., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Zhang, X., Zhang, S., Luo, G., Liu, Y. 2020. Impacts of hydraulic retention time on a continuous flow mode dual-chamber microbial fuel cell for recovering nutrients from municipal wastewater. *Science of The Total Environment*, **734**, 139220.
- Yuan, Y., Zhao, B., Zhou, S., Zhong, S., Zhuang, L. 2011. Electrocatalytic activity of anodic biofilm responses to pH changes in microbial fuel cells. *Bioresource Technology*, **102**(13), 6887-6891.
- Zhang, L., Li, C., Ding, L., Xu, K., Ren, H. 2011. Influences of initial pH on performance and anodic microbes of fed-batch microbial fuel cells. *Journal of Chemical Technology & Biotechnology*, **86**(9), 1226-1232.
- Zhuang, L., Zhou, S., Li, Y., Yuan, Y. 2010. Enhanced performance of air-cathode two-chamber microbial fuel cells with high-pH anode and low-pH cathode. *Bioresource Technology*, **101**(10), 3514-3519.

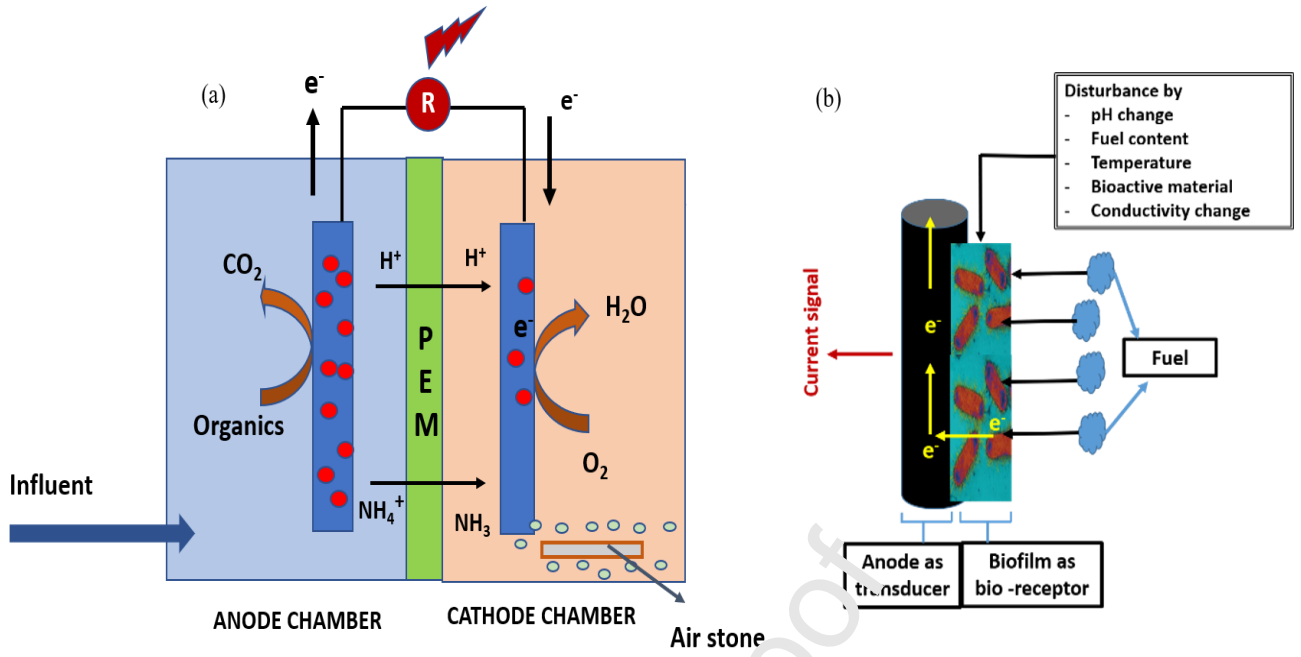


Fig. 1: (a) Microbial fuel cell system, (b) Function of MFC as a biosensor

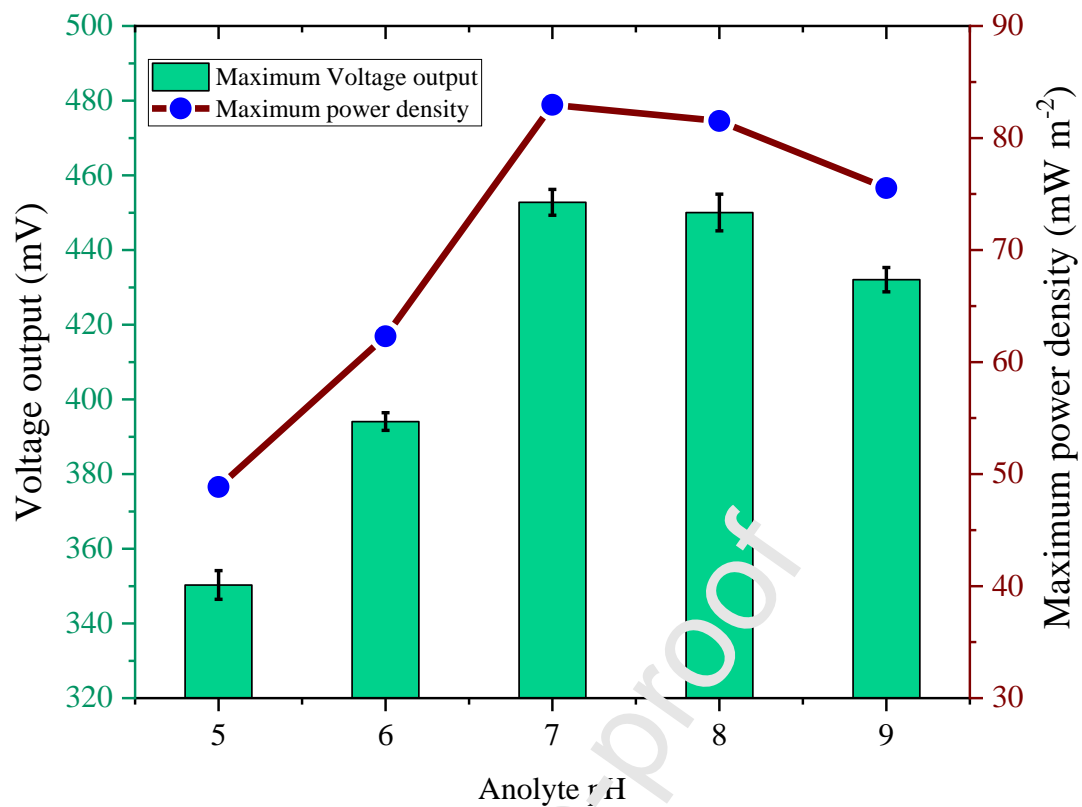


Fig 2: Maximum voltage and power density of the MFC biosensor per different pH of the anolyte. Error bars represent standard deviation

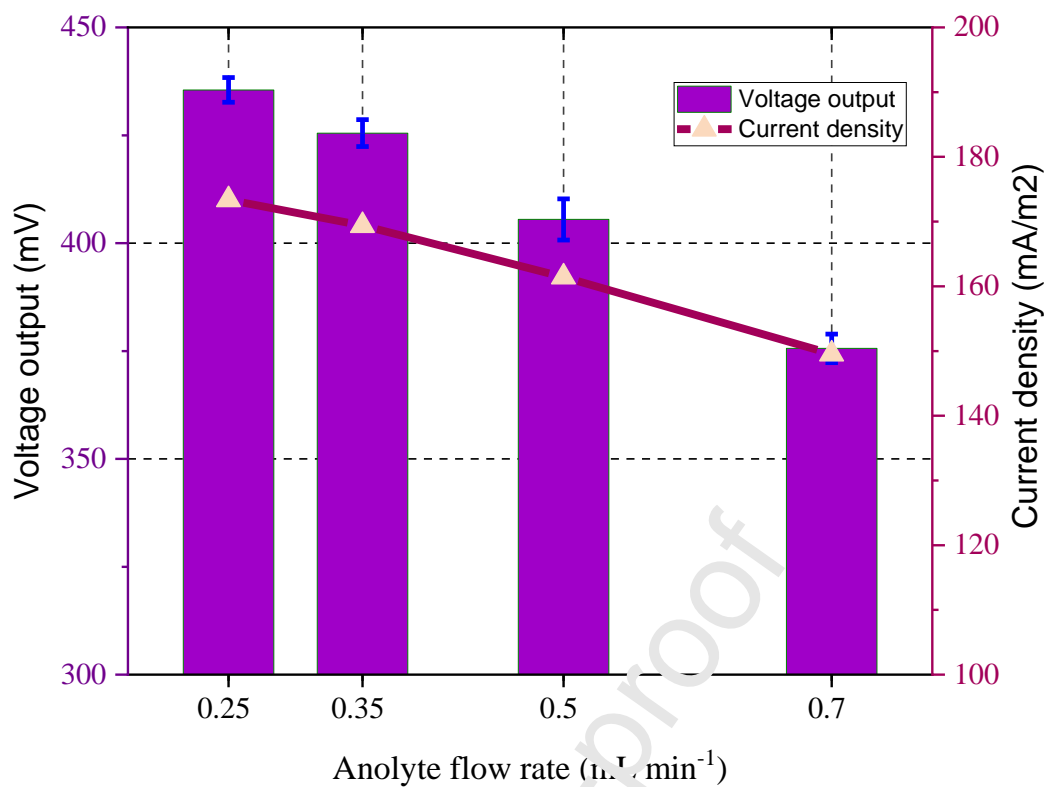


Fig 3: Voltage output and current density of MFC at a different anolyte flow. Value and error bars illustrate mean and standard deviation



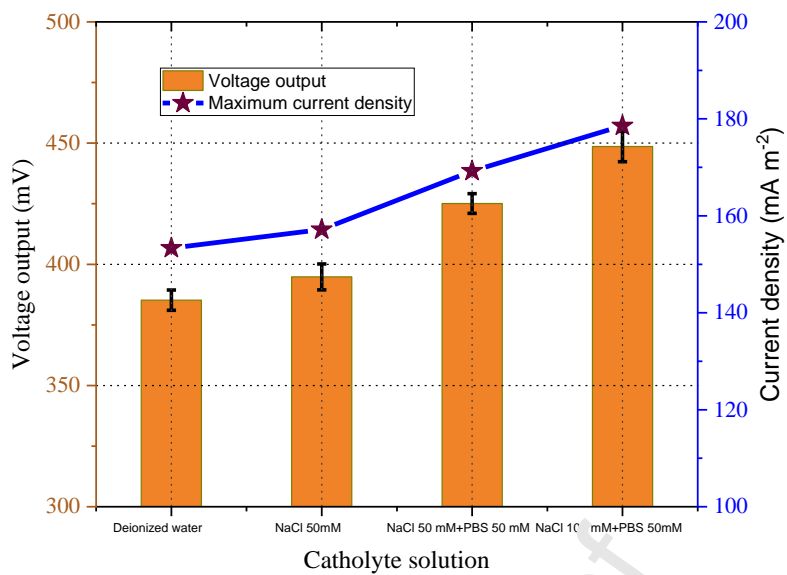


Fig 4: Current density in MFC biosensor with different components of the catholyte solution. Error bars present the standard deviation

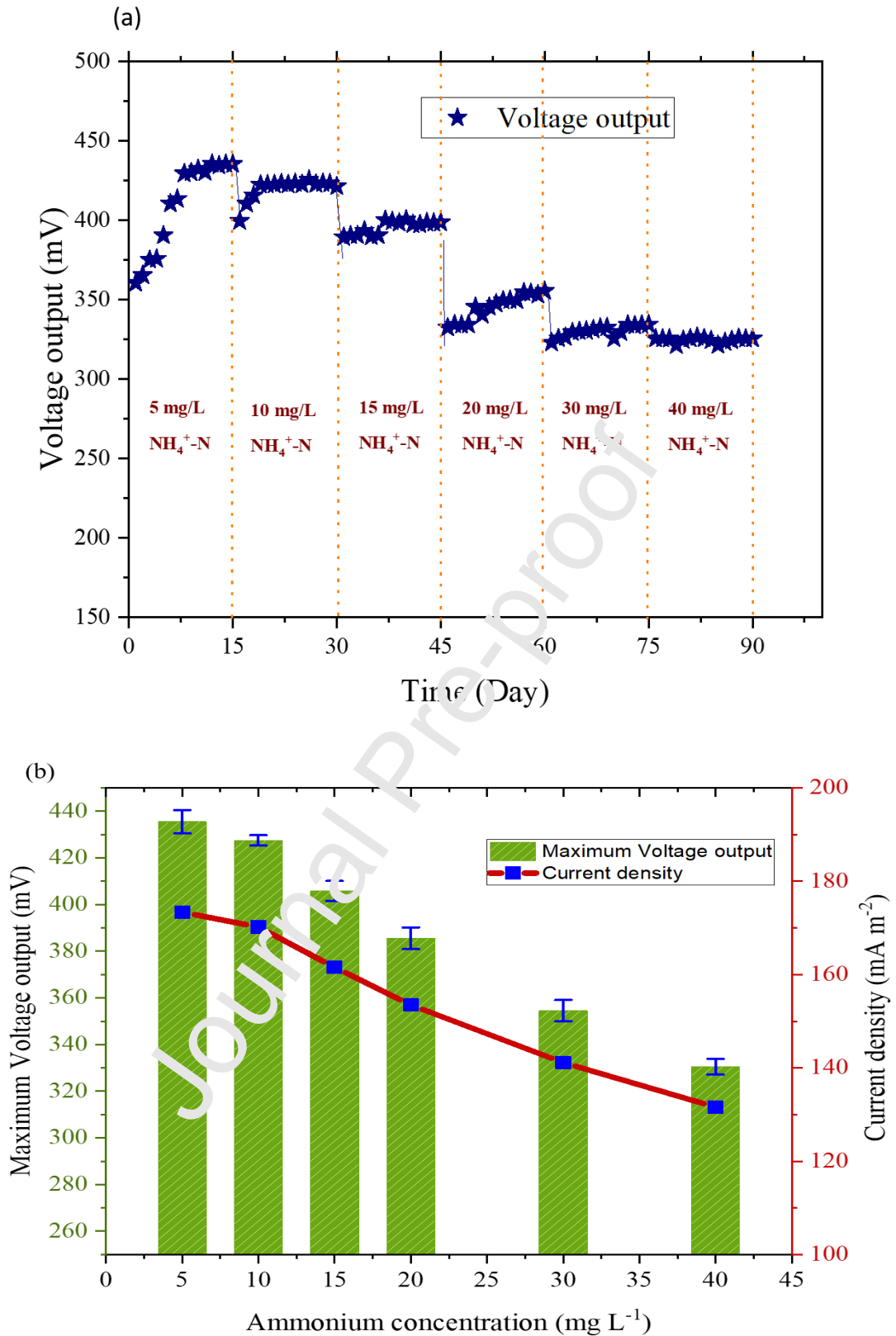


Fig 5: (a) Energy output generation with times at different influent  $\text{NH}_4^+\text{-N}$ ; (b) Maximum voltage and current density of MFC at different applied ammonium concentrations. Error bars demonstrate the standard deviation.

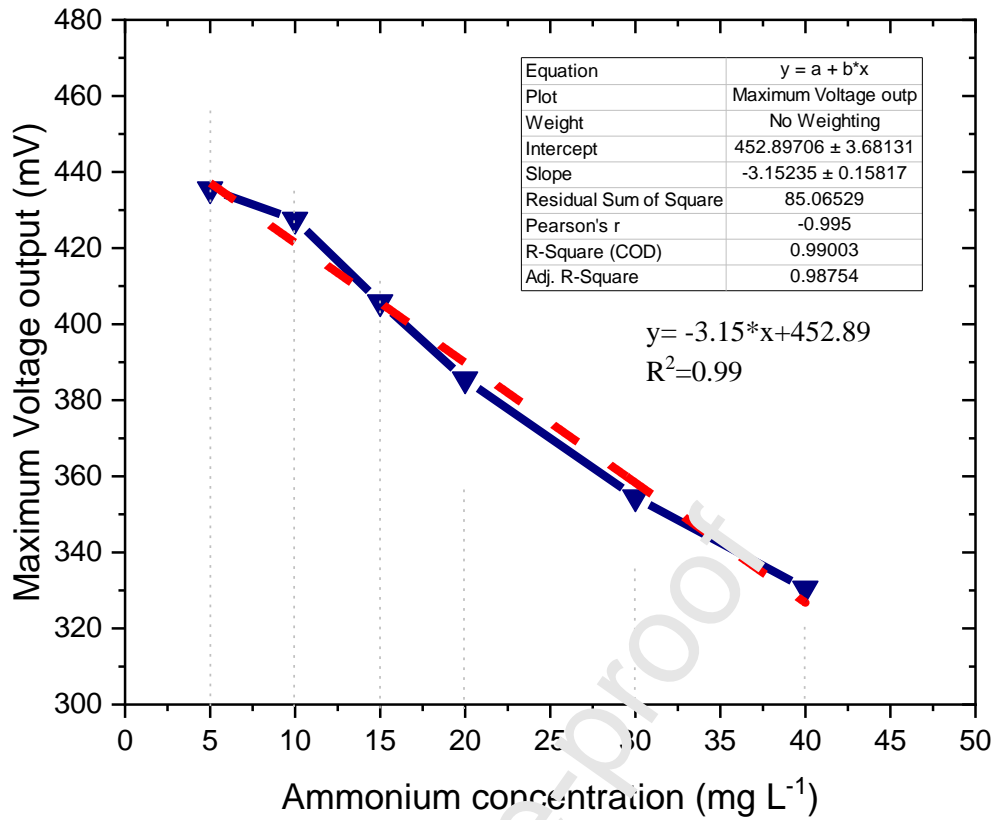
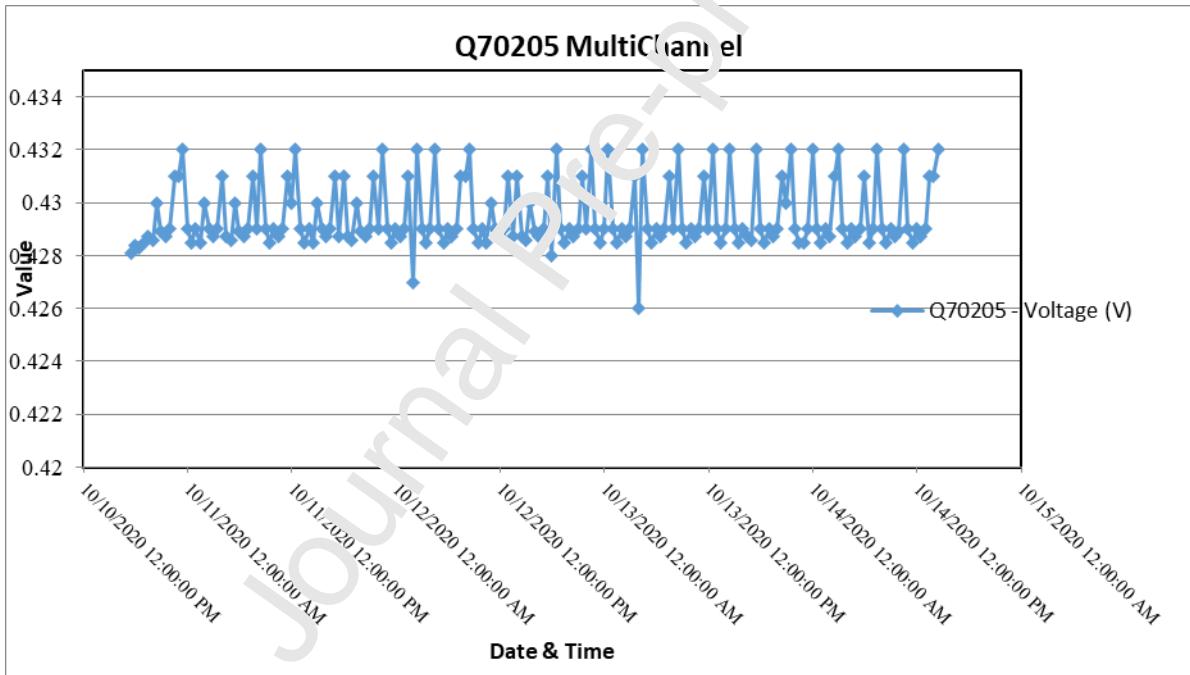
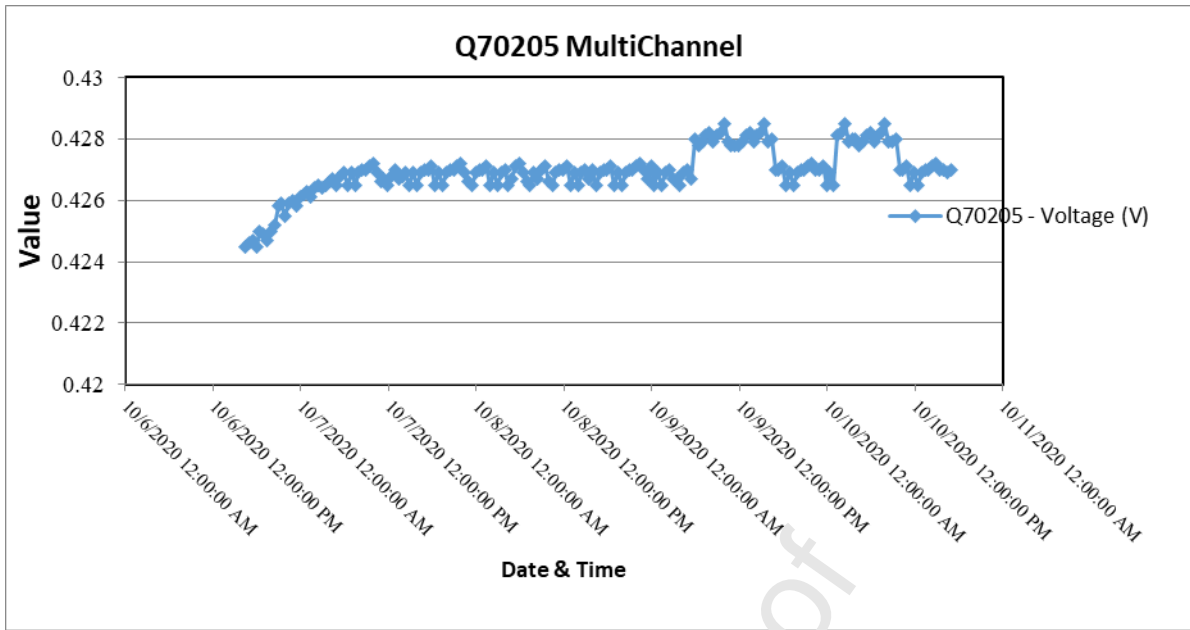


Fig 6: Relationship between the voltage output of MFC with the varying  $\text{NH}_4^+\text{-N}$



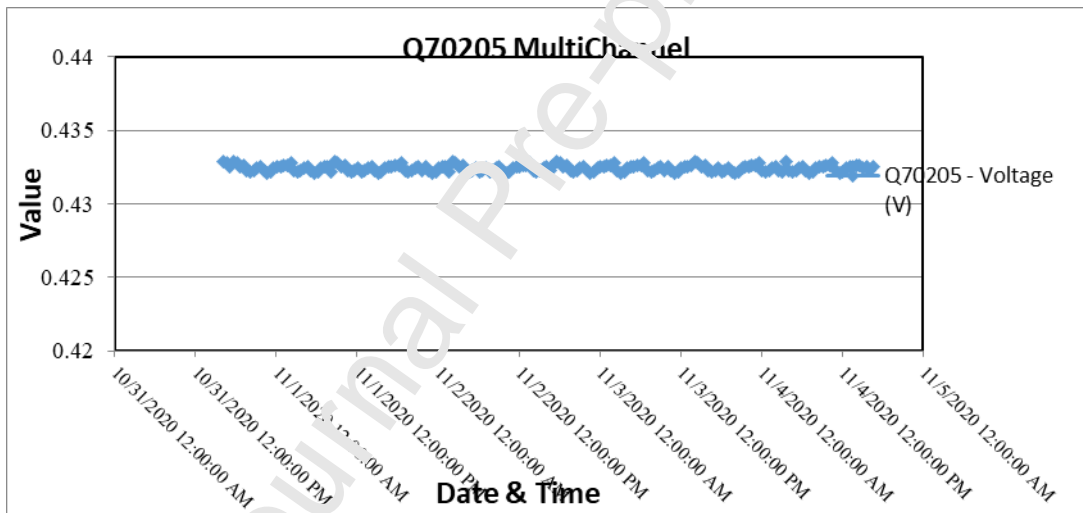
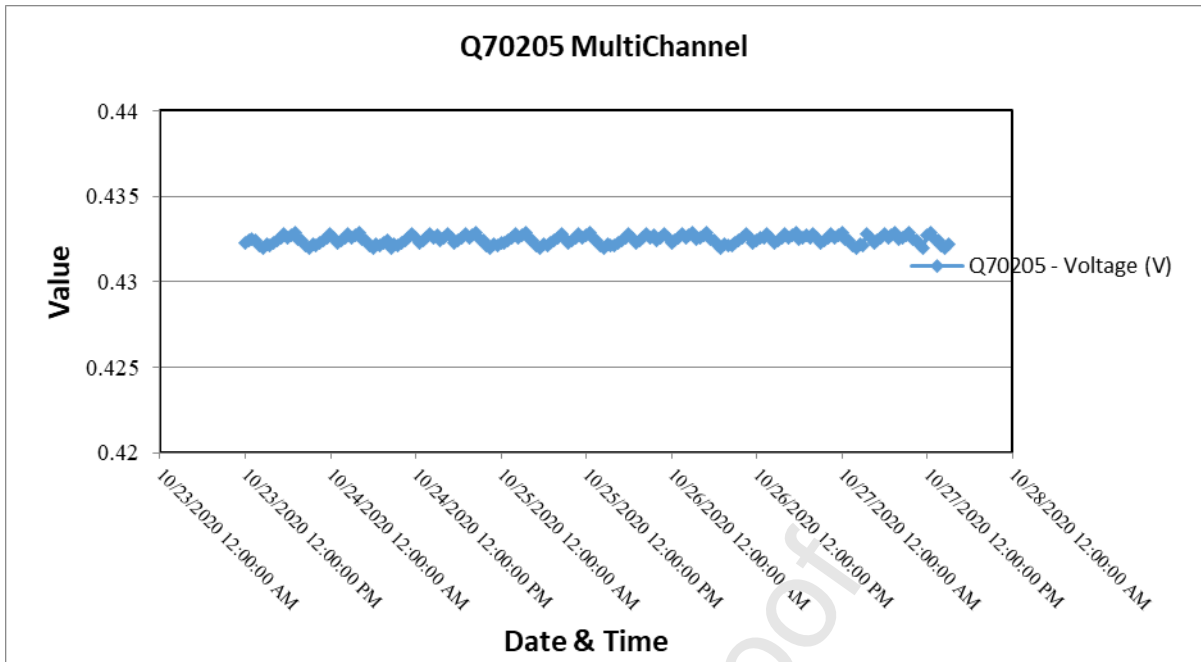


Fig 7: Data of cell voltage in 30 days

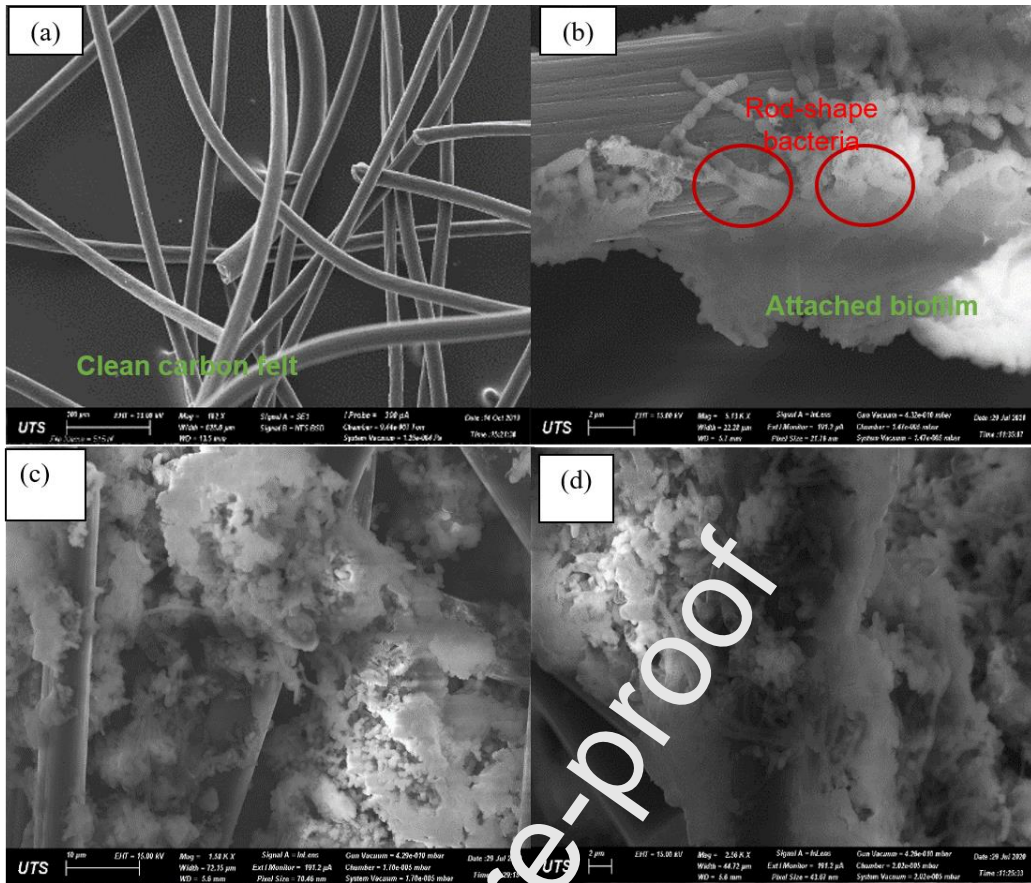


Fig. 8: SEM images of biofilms formation on the surface of the carbon felt, (a): Carbon felt before the experiment ; (b, c, d): Carbon felt with the attached biofilm at different observation

**Table 1:** Operation parameters

Parameters	Experimental process					
	1	2	3	4	5	6
Days	1-15	16-30	31-45	46-60	61-75	76-90
NH <sub>4</sub> <sup>+</sup> - N (mg/L)	5	10	15	20	30	40
HRT (h)	24	24	24	24	24	24
OLR (mgCOD/L.d)	300	300	300	300	300	300

**Table 2:** Comparison measurement of ammonium concentration

Ammonium concentration		Relative error (%)	
MFC biosensor	Test kit		
5	5.03±0.04	5.02±0.09	0.39
10	9.98±0.07	9.92±0.19	0.60
15	15.5±0.12	15.08±0.25	2.70
20	20.64±0.7	20.24±0.70	1.94
30	31.53±0.76	31.25±0.87	0.89
40	41.45±0.98	40.69±1.43	1,83

**Highlights**

- Ammonium detection in wastewater is well achieved by a dual chamber MFC biosensor.
- Relation between  $\text{NH}_4^+$  concentration and voltage generation was inversely linear.
- The dual chamber MFC biosensor could detect up to  $40 \text{ mg L}^{-1}$  of  $\text{NH}_4^+$  -N.
- Excess ammonium inhibits the activity of electrogenic bacteria in the anode chamber.
- Optimal operating conditions for the MFC biosensor are pH 7,  $1000 \Omega$  and 24-hr HRT.

Journal Pre-proof