Malaysian Journal of Microbiology, Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Bioelectricity generation using banana peel as substrate in dual-chamber *Pseudomonas aeruginosa* based microbial fuel cell

Normaiza Nordin[2], Zarina Amin[1]* and Sazmal Effendi Arshad[2]*

[1]Biotechnology Research Institute, Universiti Malaysia Sabah.
[2]Faculty of Science and Natural Resources, Universiti Malaysia Sabah.
Email: zamin@ums.edu.my; sazmal@ums.edu.my

ABSTRACT

Aims: Banana peel (BP) waste is still underutilized in Malaysia, which can be used as source of renewable energy. Microbial fuel cell (MFC) is a device that utilizes biomass to convert chemical energy into electrical energy with help of the microbial catalysis. The present study evaluates the current generation of MFC supplemented with BP waste as substrate for *Pseudomonas aeruginosa* ATCC 27853.
Methodology and results: The CHNS result shows that the C:N ratio of BP is 27:1 which is within the optimum C:N ratio for the microbial food requirement. Fluctuation of current increases as concentration of banana peel extract (BPE) decreases from 1:10, 1:20, 1:40 and 1:80, thus making 1:10 BPE optimum. Current fluctuation is related to microbial activity due to the sufficiency of nutrients which subsequently affect the performance of MFC. BPE and banana peel slurry (BPS) comparison shows that BPS is optimum. BPE reaches a maximum current of 3.91 µA in ascending phase which is higher compared to BPS (3.65 µA). In descending phase, BPE current drops to 2.31 µA compared to 2.98 µA of BPS. In stationary phase, BPS able to maintain a higher current compared to BPE. MFC maximum current was doubled to 6.52 µA when PEM was treated priorly.
Conclusion, significance and impact of study: Besides exploring and improving the ability of MFC as an alternative for power production other than fossil fuel, this research also encourage society to fully utilize waste as a source of renewable energy instead of throwing it into garbage without productivity.

Keywords: Banana peel, bioelectricity, current generation, microbial fuel cell, *Pseudomonas aeruginosa* ATCC 27853

INTRODUCTION

Malaysian's government have introduced the National Energy Policy (NEP) 2022-2040 with the objectives of enhancing macroeconomic resilience and energy security, achieving social equitability and affordability, and ensuring environmental sustainability. This study goes hand in hand with the government aspiration which is to transition to a low carbon nation. Part of this aspiration targeted in the increment in percentage of residential energy efficiency saving, increment in total installed capacity of renewable energy, reduction of coal in installed capacity and increment in percentage of renewable energy in total primary energy supply. Along with other renewable energy technology such as solar, wind, hydro and biofuels, microbial fuel cells (MFC) have attracted researchers' interest since it can harness cleaner electricity directly from organic waste. MFC is a bioelectrochemical system that utilizes microorganisms as biocatalyst to convert chemical energy into electrical energy (Logan *et al.*, 2019). Electron and proton generated in MFC is a result from oxidation of organic substance by microorganisms (Logan *et al.*, 2006). Production of electron and proton occurred in anode compartment when the bacterial species oxidizes the organic matter. Organic matter that was used as substrate in MFC, work as a carbon source for microbes and also an electron donor in the metabolic pathway (Idris *et al.*, 2022). It can be obtained from various sources and one of them is food waste. In Malaysia, the amount of unconsumed food being thrown away is 16,688 tonnes per day and nearly 80% of the waste are disposed at landfill (Hashim *et al.*, 2021). Food waste will become a threat if not managed and treated properly. Even though the unconsumed food is considered as waste by human, they are actually a beneficial source of nutrient for microorganisms (Yaqoob *et al.*, 2022). Another study reported by Phooi *et al.* (2022) in understanding Malaysian's awareness on food waste behaviour and food waste component for decomposition, shows that 61% of the respondent discarded organic food waste which come from the part of fruit and vegetable that cannot be consumed. There are lot of studies that have been reported on using organic food waste as nutrient for the growth of microbes in MFC. Jenol *et al.* (2019) utilizes both unhydrolyzed and hydrolysed sago pith waste as

***Corresponding author**

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

substrate and have obtained a maximum power output of 73.8 mW/m^2 and 56.5 mW/m^2, respectively. Priya and Setty (2019) reported a maximum power output 31.58 mW/m2 when using cashew apple juice as substrate. Sugarcane molasses have also been used previously by Hassan *et al.* (2019) producing 188.5 mW/m^2. Other than that, Makhtar and Tajarudin (2020) have reported on using corn barn, palm oil mill effluent and banana peel with maximum power output of 12.65 mW/m^2, 22.03 mW/m^2 and 23.75 mW/m^2, respectively. The latest study carried out by Rojas-Flores *et al.* (2023) in which tangerine waste was supplemented in their MFC producing maximum power output of 475.32 mW/cm^2. Utilizing organic food waste in MFC is an alternative to produce green energy other than being overly dependent on fossil fuel. Banana is an important fruit in Malaysia. According to Tan (2022), in 2020 bananas production was recorded to be 312,968 tonnes. Thirty to forty percent of the total weight of the whole banana belong to the peels (Zahrim *et al.*, 2015), which contribute to an increase in unutilized organic waste (Basirun *et al.*, 2023). It contains a lot of valuable nutrition and can be easily obtained from places like production factories, households, restaurants, and marketplaces. Banana peel waste rich in carbohydrates, proteins, phenolic compounds, macro and micronutrients (Verma and Mishra, 2022). Besides that, in dry matter basis, banana peel contains 14.6% glucose, 56% sucrose, 6 to 9% protein, 20% fibres and 7 to 5% starch (Sulong *et al.*, 2021). In this study, *P. aeruginosa* ATCC 27853 was inoculated in dual-chamber microbial fuel cell (DC-MFC) containing banana peel substrate to produce current, with the objectives (1) to develop an optimized DC-MFC using *P. aeruginosa* and banana peel substrate, (2) to compare current output between four different concentration of banana peel extract and its relation with biofilm formation, (3) to compare the performance of MFC with different form of banana peel substrate and (4) to explore the effect of proton conductivity on current output for pretreated proton exchange membrane.

MATERIALS AND METHODS

Substrate collection and preparation

Banana peel waste was collected, and the substrate was prepared into two form which are banana peel extract (BPE) and banana peel slurry (BPS).

Pretreatment of banana peel

Banana peel was washed under running water to remove dust and other contaminant. It was then subjected to oven drying at 60 °C, then ground into powder form. Powder form banana peel was then sieved and kept in airtight plastic bag. It was stored in 4 °C until further use.

Preparation of banana peel extract

BP content was extracted using water. Four concentrations ratio of banana peel powder to water was prepared using the following ratios: 1:10, 1:20, 1:40 and 1:80. The mixture was left macerated on the orbital shaker with rotation speed 200 rpm for 24 h. BPE was obtained by filtering the supernatant using cheese cloth and discarding the solid part. The extract was then sterilized using autoclaved with temperature of 121 °C, at 15 psi for 15 min.

Preparation of banana peel slurry

100 g of washed banana peel was added into an electronic blender and pulverized with 1 L of distilled water. The BPS was then autoclaved at 121 °C, 15 psi for 15 min, for sterility purpose.

Substrate characterization

Elemental analysis of banana peel was carried out using CHNS Analyzer (Vario MACRO cube) to determine carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) content and to calculate the carbon to nitrogen ratio.

Assembly of microbial fuel cell

DC-MFC was constructed using acrylic Perspex sheet with measurement of 5 mm thickness and total volume of 512 cm^3 for each compartment as shown in Figure 1. DC-MFC was designed to be detachable, thus making it easier to reuse the proton exchange membrane (PEM). Figure 2 shows the schematic diagram of a complete setup of the system. The DC-MFC consist of anode and cathode compartment, in which anode and cathode compartments were complimented with stainless steel mesh and graphite rod electrode respectively. The surface area of stainless steel mesh is 40 cm^2 and 10 cm^2 for graphite rod. The anode and cathode compartment were separated with PEM (Nafion^{TM} 117, USA) with diameter of 3 cm. PEM helps preventing intermixing of solution in both compartments and allow the movement of proton from anode to cathode (Miran *et al.*, 2016).

Pretreatment of PEM

Pretreatment of Nafion membrane is according to Ghasemi *et al.* (2013), with slight modification. Table 1 summarizes the steps for PEM pretreatment. Two types of acids were used which are 3% v/v hydrogen peroxide (H_2O_2) and 0.5 M sulfuric acid (H_2SO_4). The Nafion membrane was heated in the water bath at 100 °C. Firstly, it was heated for an hour in distilled water and then followed 3% v/v H_2O_2 and 0.5 M H_2SO_4. In between of the two acids treatment, the PEM was washed using distilled water. Treated PEM was then stored in distilled water until further used to prevent membranes from swelling (Chae *et al.*, 2008).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

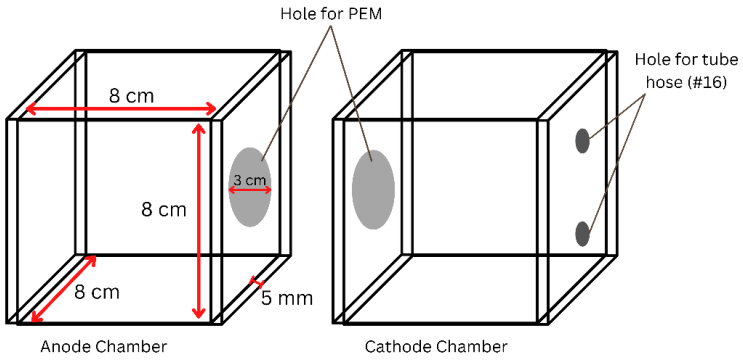

Figure 1: Schematic diagram of DC-MFC showing the measurement of the anode and cathode chamber.

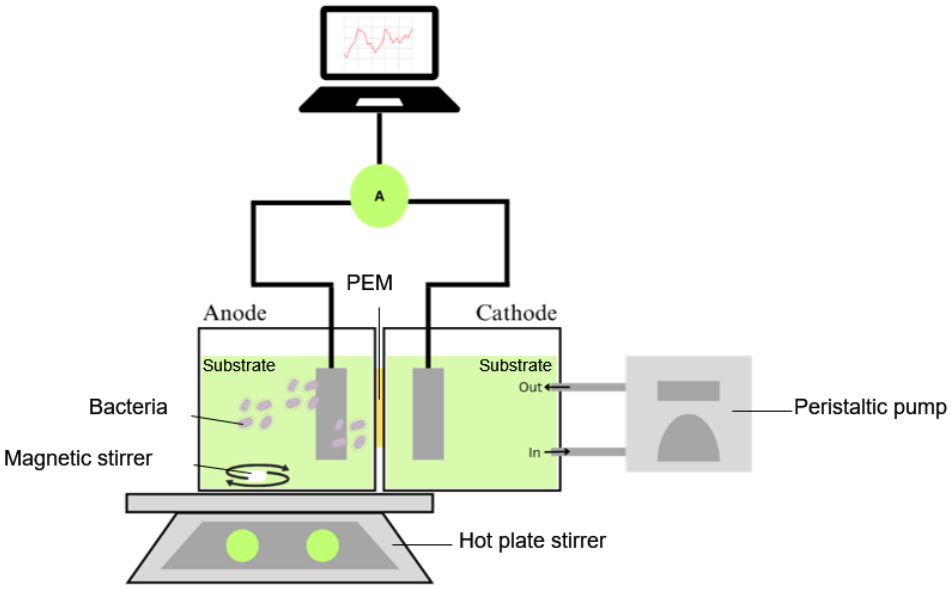

Figure 2: Schematic diagram of the complete setup of DC-MFC system.

Table 1: Pretreatment of Nafion 117 membranes.

Time	Concentration	Reagent	Water bath temperature(°C)
60 min		Distilled water	100
60 min	3%	H_2O_2	100
60 min		Distilled water	100
60 min	0.5 M	H_2SO_4	100
60 min		Distilled water	100

Preparation of bacterial suspension

Pseudomonas aeruginosa ATCC 27853 was obtained from the culture collection of Biology Research Institute of Universiti Malaysia Sabah. To grow and maintain the *P. aeruginosa* stock, the original stock was first revived on tryptic soy agar by aseptically streaked one loop full of these bacteria on the agar and incubated it overnight. Next, the bacteria were cultured in tryptic soy broth overnight. Pure glycerol was added into the liquid culture of *P. aeruginosa* and kept in -80 °C until further use. The presence of glycerol will help stabilizing the frozen bacteria, as it keeps the cells alive by preventing damage to the cell membrane when stored in -80 °C (Islam, 2020).

Preparation of 0.5 McFarland standard solution

McFarland standard solutions are used to standardize the approximate concentration of bacteria in a liquid

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

Table 2: Amount of $BaCl_2$ and H_2SO_4 required to prepare different concentration of McFarland standard.

McFarland standard	1% $BaCl_2$ (mL)	1% H_2SO_4 (mL)	Colony-forming units per millilitre (CFU/mL)
0.5	0.05	9.95	1.5×10^8
1.0	0.10	9.9	3.0×10^8
2.0	0.20	9.8	6.0×10^8
3.0	0.3	9.7	9.0×10^8
4.0	0.4	9.6	1.2×10^9
5.0	0.5	9.5	1.5×10^9
6.0	0.6	9.4	1.8×10^9
7.0	0.7	9.3	2.1×10^9
8.0	0.8	9.2	2.4×10^9
9.0	0.9	9.1	2.7×10^9
1.0	1.0	9.0	3.0×10^9

Figure 3: Black and white stripes used as background to compare the turbidity of the bacterial suspension in phosphate buffer saline with 0.5 McFarland standard.

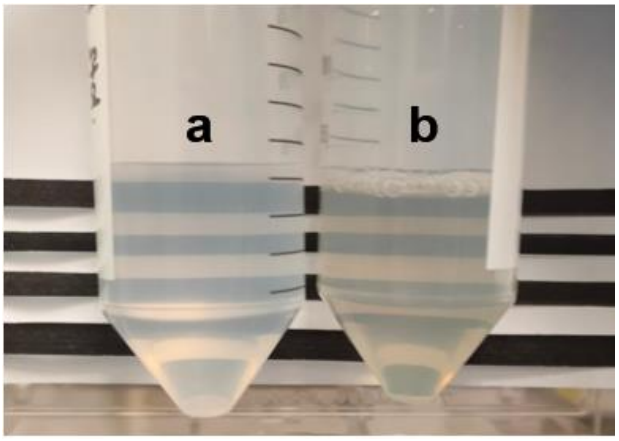

Figure 4: (a) 0.5 McFarland standard solution; (b) Bacterial suspension.

suspension by visually comparing the turbidity of prepared bacterial suspension with McFarland standard. Table 2 shows the amount of $BaCl_2$ and H_2SO_4 required to prepare the desired approximate concentration of colony-forming units of bacteria per millilitre (CFU/mL) (Zapata and Ramirez-Arcos, 2015). In this study an approximate amount of 1.5×10^8 CFU/mL was used as an initial concentration in MFC during the whole experiment. Thus 0.5 McFarland standard solution was prepared by mixing 0.05 mL of 1% $BaCl_2$ into 9.95 mL of 1% H_2SO_4. To compare the turbidity of bacterial suspension in phosphate buffer saline and the McFarland standard, the black and white stripe shown in Figure 3 was used as background. To prepare an approximate bacterial suspension of 1.5×10^8 CFU/mL, frozen stock of bacteria was first thawed and pipetted into the diluted PBS until the turbidity is identical to 0.5 McFarland standard solution as shown in Figure 4. Adjustment was made by adding more bacteria to increase the turbidity or adding more PBS to dilute the turbidity. Bacterial suspension in phosphate buffered saline was prepared before operating the MFC and used instantly after preparation.

MFC operation

Experiment was done in three stages to find the optimum conditions of each parameter. First stage is to find the optimum concentration of substrate. Four concentrations of 1:10, 1:20, 1:40 and 1:80 BPE was involved, and each concentration was operated for two weeks. Second stage is to find the optimum form of substrate, which is between BPE and BPS, and the third stage is to identify the optimum condition of PEM, either untreated or pretreated. Each MFC operation was left running for two weeks as in stage one. Banana peel substrate served as the primary source of nutrient for the microbial growth and no additional nutrient was added in the system. Utilization of banana peel substrate alone will reduce the complexity of the systems, thereby portraying the actual potential of banana peel waste as carbon source in MFC. To allow movement of substrate, anode compartment was stirred using magnetic stirrer at 150 rpm, meanwhile substrate in cathode was pump using peristaltic pump with a flow rate of 150 rpm. Current was recorded every 1 min using a digital multimeter (UNI-T, model UT61E+, China) which was connected to a personal computer to record the cell performance. To make sure the MFC was run in sterile condition and no other microbes involved other than introduced microbes, experiment was carried out in laminar flow cabinet. All equipment was sanitized with 75% ethanol and exposed to UV radiation. Before introducing *P. aeruginosa* into the MFC, sterility test was carried out by pipetting out 10 µL of substrate from both chamber and streak on tryptic soy agar to ensure the MFC is sterile. After incubating the agar overnight, if there is no sign of contamination, the system was left running for two weeks, otherwise repetition was carried out.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

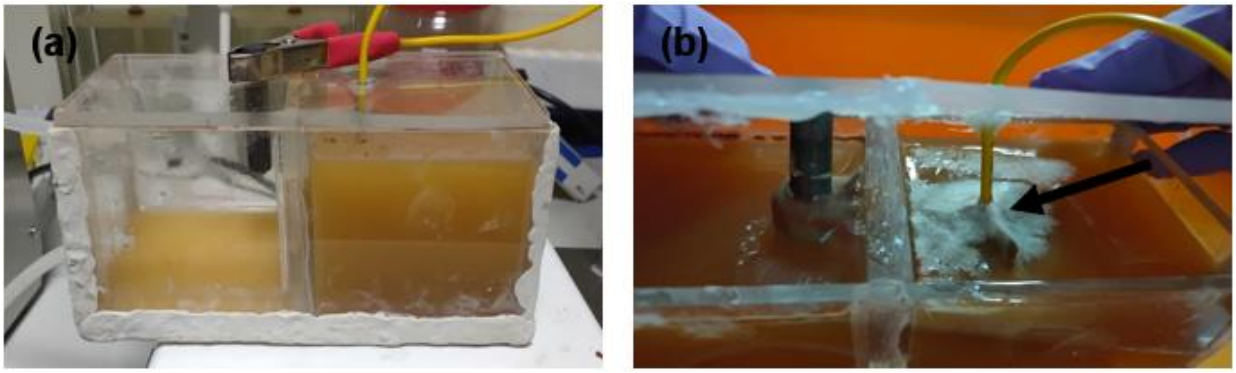

Figure 5: (a) Initial DC-MFC setup encounter leaking issues; (b) Arrow pointing on the contamination of substrate after two weeks of operation.

MFC characterization

The recorded current was plotted against time in days.

Scanning electron microscopy (SEM) analysis

SEM analysis was carried out to study the surface morphology of stainless steel mesh electrode with and without formation of biofilm to support the current output of DC-MFC. SEM analysis was done to observe the formation of biofilm on anode electrode. After running the MFC for 2 weeks, the anode electrode was taken out and washed with phosphate buffer three times to remove contaminant. Fixation was carried out by immersing anode electrode for an hour at room temperature in 2.5% glutaraldehyde. Next, the electrode was gradually washed using 30, 50, 70, 80, 90 and 99% then followed by air dry. Electrode was sputtered with gold ion for 120 sec to improve the resolution of the image (Thapa and Chandra, 2019).

RESULTS AND DISCUSSION

CHNS analysis result

Table 3 shows the carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) percentage (%) of banana peel in dry matter basis. Verma and Mishra (2022) reported a comparable amount of elemental composition in dried banana peels which makes it suitable to be used as source of nutrient for microbial growth. C:N ratio from the CHNS result in this study was calculated to be 27:1. According to Saleh *et al.* (2012), 20-30:1 is the optimum C:N ratio of microbial food requirements.

MFC integrity

The integrity of assembled MFCs was maintained by establishing the preliminary data that can be used for further improvement. During the preliminary study, few issues rose which include, MFC leakage (Figure 5a), substrate contamination (Figure 5b) and un-reusable setup resulting in higher cost requirement. These issues cause inaccuracy in data collection. The issues were tackled by redesign the MFC to make it reusable and to prevent leakage (Figure 6). On the other hand, contamination issue was solved by sterilizing all equipment using 75% ethanol and UV radiation in the beginning of experiment and running MFC in laminar flow cabinet to provide more sterile air circulation.

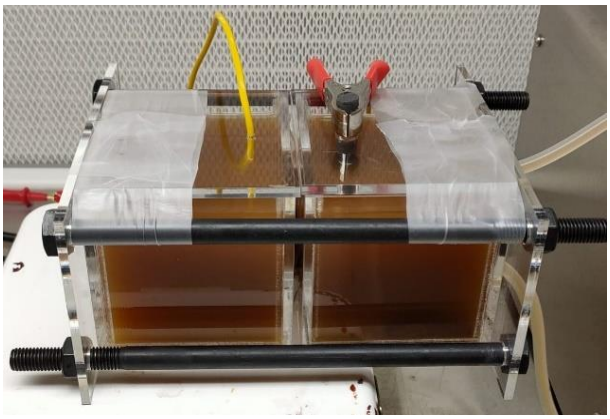

Figure 6: Improved version of DC-MFC.

Table 3: CHNS analysis of banana peel powder.

Element	Amount (%)
C	37.09
H	4.605
N	1.36
S	0.073
Unknown	56.872

Analysis of current generation of MFC

Current generation of different concentration of BPE with P. aeruginosa

The power generation shown in Figure 7a-d was observed for two weeks, and it was analysed based on the current fluctuation and the four phases of current

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

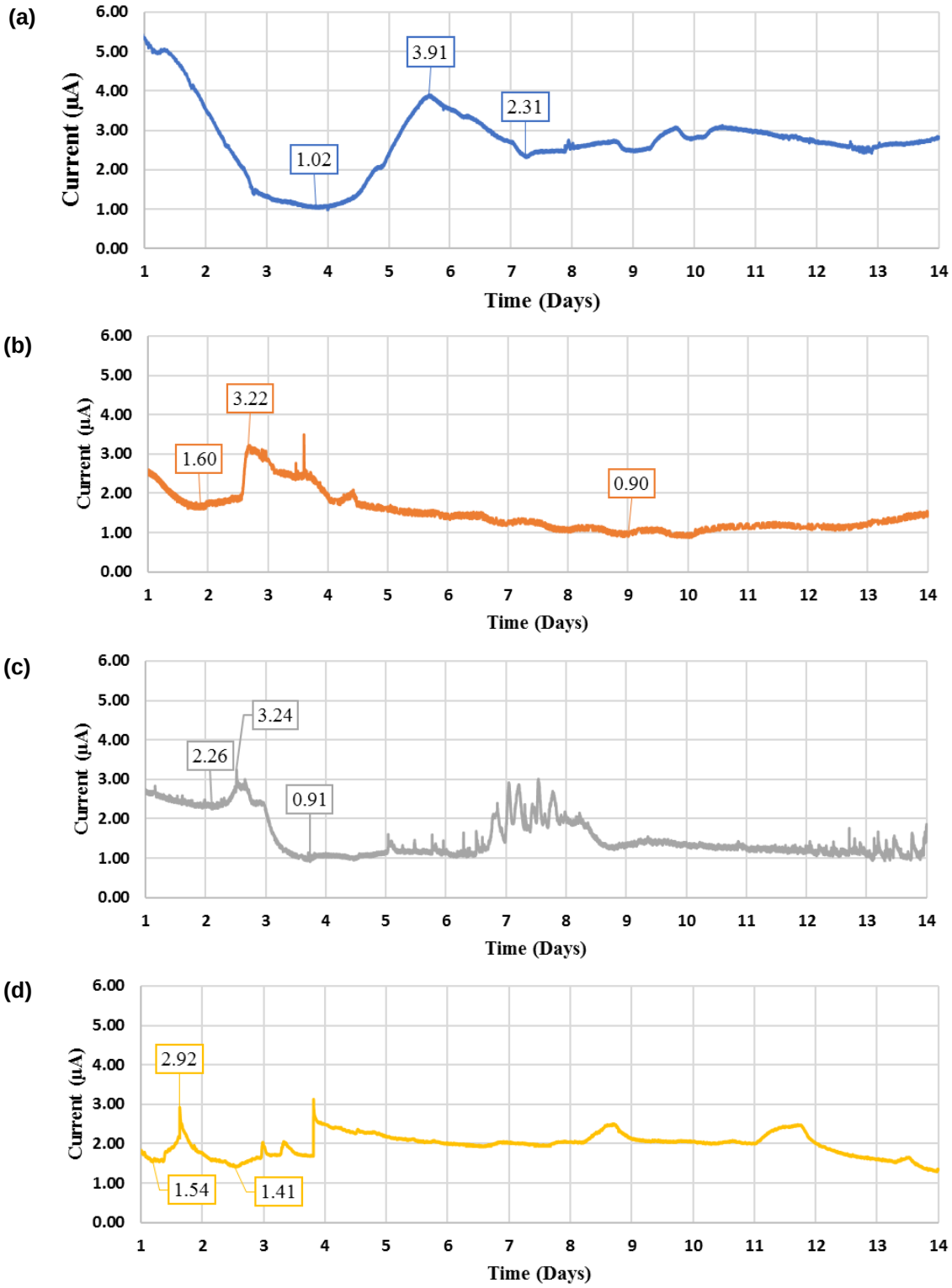

Figure 7: (a) Current generation of DC-MFC after two weeks of operation with 1:10 BPE; (b) Current generation of DC-MFC after two weeks of operation with 1:20 BPE; (c) Current generation of DC-MFC after two weeks of operation with 1:40 BPE; (d) Current generation of DC-MFC after two weeks of operation with 1:80 BPE.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

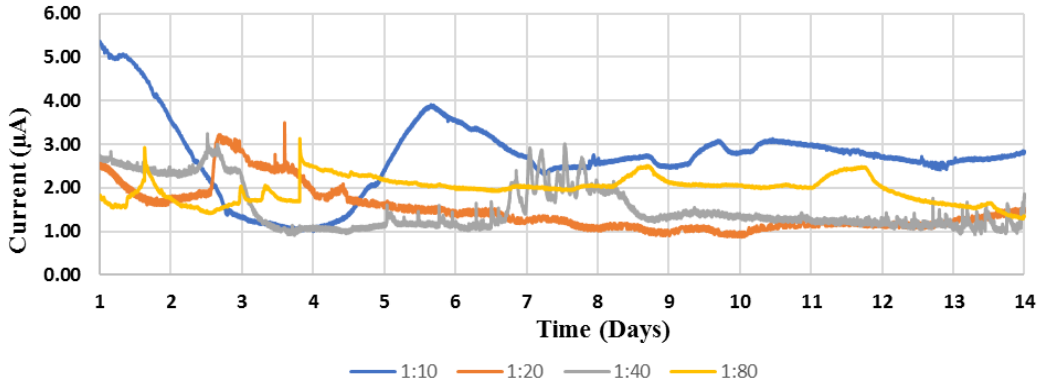

Figure 8: Current generation of DC-MFC of different concentration of BPE after two weeks of operation showing different level of fluctuation.

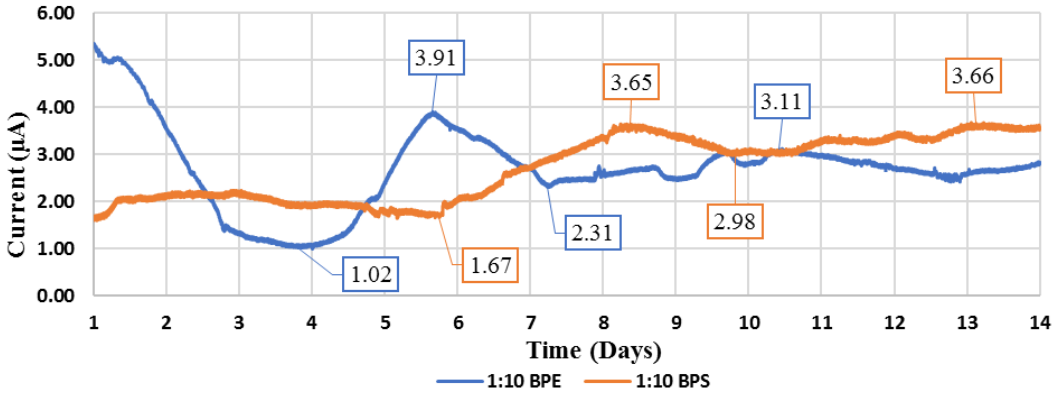

Figure 9: Current generation of DC-MFC of 1:10 BPE and 1:10 BPS after two weeks of operation.

generated which involve, adapting phase, ascending phase, descending phase and stationary phase. Current output was plotted against time (days). Adapting phase can be seen in the beginning of the graph which shows a decrement indicating that bacteria is still in the process of adapting to the new environment (William *et al.*, 2019). Yaqoob *et al.* (2021) mentioned that, during this phase, electrocatalytic activities is low due to the poor bacteria densities on anode surface. Ascending phase started when current generated increases after the first lowest current was recorded during adapting phase. 1:10 BPE was observed to have the highest peak (3.92 µA) during ascending phase followed by 1:40 (3.24 µA), 1:20 (3.22 µA) and 1:80 (2.92 µA). According to Rojas-Flores *et al.* (2021b), biofilm that was formed on anode electrode, contained enzymes that pulled the electrons that was released during metabolisms of substrate by microbes which subsequently generate changes in current generation. It is also an indication to the formation of biofilm on anode electrode (Krithika *et al.*, 2021).

Descending phase started when current generated decreases after the first peak was recorded. This phase might be due to deterioration of biofilm performance because of inhibition of electron transfer from the bacteria to the anode surface by the matured bacterial biofilm (Majumder *et al.*, 2014). Depletion of nutrient contain in substrate also leading to decrement of current value (Rojas-Flores *et al.*, 2023). In 1:10 BPE, the current drops to 2.31 µA. Comparing to 0.90 µA (1:20 BPE), 0.91 µA (1:40 BPE) and 1.41 µA (1:80 BPE), 1:10 BPE generated the highest current during this phase. Stationary phase is an additional phase that was used to compare how much current was able to maintain until the end of MFC operation. Between all four concentration, 1:10 BPE able to maintain the current generated in the range of 2 µA up to 3.11 µA. Meanwhile, other concentration only able to maintain the current less than 3 µA. The production of power in MFC is caused by microbial activity. But over time, microbial activity may become unstable and fluctuated, which will affect how much power is produced. This instability might be due to low nutrient availability (Koffi and Okabe, 2020). Based on Figure 8, concentration that shows least fluctuation in current output is 1:10 BPE, which is the MFC with highest concentration. As the concentration reduces the current fluctuation getting more significant. This result shows that the current fluctuation observed in this study was related to unstable microbial activity due to the nutrient availability.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

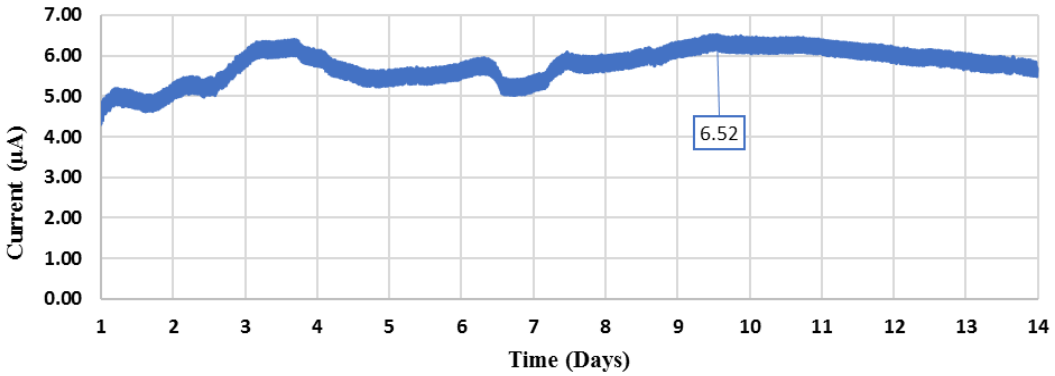

Figure 10: Current generation of DC-MFC supplemented with 1:10 BPS shows an increment when PEM was treated priorly before used.

Current generation of different form of substrate

According to Rojas-Flores *et al.* (2021a), the rate of substrate breaks down and concentration of complex organic compound can affect the electricity generation. If either rate of material breaks down and/or concentration of substrate is low, the electricity generation will also be low. Figure 9 shows the current generation of banana peel prepared in different form. It can also be seen from the current trend, where 1:10 BPS took more than eight days to reach the maximum current compared to 1:10 BPE that only took approximately six days from the beginning of operation. During ascending phase,1:10 BPE shows a higher maximum current (3.91 µA) compared to 1:10 BPS (3.65 µA). Higher current output of 1:10 BPE during ascending phase might be due to higher rate of substrate breakdown in banana peel extract compared to banana peel slurry. However, in descending phase, current output of 1:10 BPE drop to 2.31 µA which is lower compared to 1:10 BPS (2.98 µA). Even though 1:10 BPE recorded a higher maximum current, 1:10 BPS able to maintain higher current starting from descending phase until the end of the operation. In addition, 1:10 BPS shows a more stable current compared to 1:10 BPE. Higher current drop on the 8th day for 1:10 BPE might be due to depletion of available nutrient (Rojas-Flores *et al.*, 2021b). Higher current output for 1:10 BPS during stationary phase indicating that nutrient available during that phase is higher compared to 1:10 BPE during the same phase. Therefore, method of substrate preparation is an important factor to consider when operating an MFC.

Current generation of MFC with treated PEM

The obtained result in Figure 10 shows that the maximum current output of MFC reaches 6.52 µA which is doubled compared to MFC with untreated PEM. Ghasemi *et al.* (2013), also reported an increment of power generation from 52.8 mW/m^2 (with untreated PEM) to 100 mW/m^2 when PEM was subjected to pretreatment. When the PEM was pretreated with acid, the crystalline phase of the polymer undergoes changes that lead to the formation of amorphous phase resulting to the formation of new or larger hydrophilic domains that facilitates the transport of protons through the membrane which consequently enhance the proton conductivity (Iriarte *et al.*, 2022). Higher current output was led by a higher proton conductivity. It is one of the factors that affect MFC performance. Higher proton conductivity means proton can move more easily across the membrane, which enhance the electrochemical reaction and consequently increase the current output (Pei *et al.*, 2018).

Biofilm analysis

A vast range of microorganisms have been found to be capable in forming biofilm (Greenman *et al.*, 2021) and transferring electron to the anode electrode (Logan *et al.*, 2019). These species are known as exoelectrogenic microorganisms. Exoelectrogenic is a term used to describe the microorganisms that can transfer electron outside the cell (Logan *et al.*, 2019). Bacteria that were accumulated on the anode electrode surface is known as biofilm. It is responsible in energy generation and transportation of electron. It also improves the interaction between anode and anolyte (Yaqoob *et al.*, 2022). The biofilm around the anode surface was microbially catalysed via oxidation to produce metabolites such as electrons, protons and carbon dioxide (CO_2) (Idris *et al.*, 2022). Biofilm are made up of 97% water, 2-3% bacteria, and 3-6% extracellular polymeric materials (EPS). The major component of the biofilm is the EPS and it contribute in formation of biofilm and electron transfer (Angelaalincy *et al.*, 2018). EPS facilitated the formation of dense monolayer of electroactive biofilm. As the culture ages, more EPS was secreted leading to a thicker biofilm. According to Ishii *et al.* (2012) microbial cell growth on the electrode facilitates electron transfer and improve the current generation. EPS helps in electron transfer by acting as attachment site for peripheral redox proteins. Peripheral redox proteins are proteins that involved in electron transfer activity in MFC. Scanning electron microscopy (SEM) analysis was carried out to study the

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(6) Special Issue 2023, pp. 617-627
DOI: http://dx.doi.org/10.21161/mjm.230006

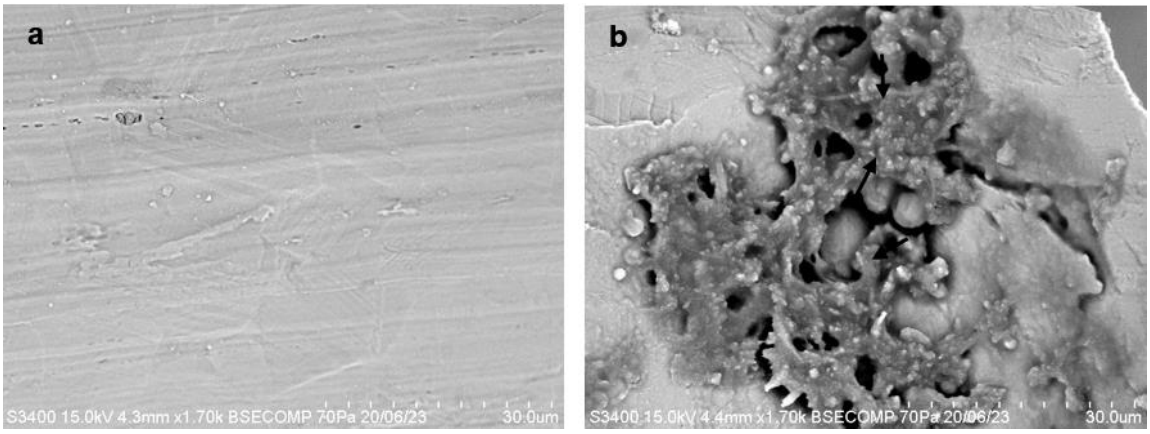

Figure 11: (a) Anode electrode without biofilm; (b) Anode electrode with *P. aeruginosa* biofilm. Arrow pointed on the EPS formation.

surface morphology of anode before MFC operation and after operation. Figure 11a is the SEM image of the stainless steel mesh anode before MFC operation and Figure 11b is the SEM image of stainless steel mesh anode after MFC operation. Stainless steel mesh anode after operation clearly shows the presence of biofilm on the anode surface. This formation explains the increment of current output reported in this study, besides supporting that *P. aeruginosa* ATCC 27853 have the ability to transfer electron outside the cell. Although the morphology of the biofilm may have been altered during dehydration process, formation of EPS can still be seen, and it was marked with arrow in the figure.

CONCLUSION

Bioelectricity was successfully generated with DC-MFC using banana peel substrate and *P. aeruginosa* ATCC 27583 with a maximum current of 6.52 µA after three stages of optimization. First stage involving substrate concentration optimization in which 1:10 BPE able to convey the best performance with maximum current of 3.91 µA. Current obtained is related to the formation of electrically active biofilm that was formed on anode electrode. The current result of 1:10 BPE was then compared with 1:10 BPS and even though 1:10 BPE have higher maximum current during ascending phase, 1:10 BPS able to maintain higher current, starting from descending phase until the end of operation. 1:10 BPS also recorded a more stable current. 1:10 BPS was chosen for the next stages of optimizing the PEM condition through acid pretreatment. Result obtained shows that PEM treatment increases the maximum current output from 3.65 µA to 6.52 µA due to greater proton conductivity. Low current might be due to low surface area of electrode, for future investigation it is recommended to use electrode with higher potential area for accommodating a microbial colony to give a higher power output. Besides that, examining substrates conductivity can provide more understanding on proliferation of microorganism for substrate optimization which subsequently affect the MFC performance.

ACKNOWLEDGEMENTS

No grant was received for this project.

CONFLICTS OF INTEREST

Authors declare no conflict of interest and all authors equally share responsibility of manuscript preparation and laboratory work.

REFERENCES

Angelaalincy, M. J., Krishnaraj, R. N., Shakambari, G., Ashokkumar, B., Kathiresan, S. and Varalakshmi, P. (2018). Biofilm engineering approaches for improving the performance of microbial fuel cells and bioelectrochemical systems. *Frontiers in Energy Research* **6, 63.**

Basirun, A. A., Othman, A. R., Yasid, N. A., Abd Shukor, M. Y. and Khayat, M. E. (2023). A green approach of utilising banana peel (*Musa paradisiaca*) as adsorbent precursor for an anionic dye removal: Kinetic, isotherm and thermodynamics analysis. *Processes* **11(6), 1611.**

Chae, K. J., Choi, M., Ajayi, F. F., Park, W., Chang, I. S. and Kim, I. S. (2008). Mass transport through a proton exchange membrane (Nafion) in microbial fuel cells. *Energy and Fuels* **22(1), 169-176.**

Ghasemi, M., Wan Daud, W. R., Ismail, M., Rahimnejad, M., Ismail, A. F., Leong, J. X. *et al.* (2013). Effect of pre-treatment and biofouling of proton exchange membrane on microbial fuel cell performance. *International Journal of Hydrogen Energy* **38(13), 5480-5484.**

Greenman, J., Gajda, I., You, J., Mendis, B. A., Obata, O., Pasternak, G. *et al.* (2021). Microbial fuel cells and their electrified biofilms. *Biofilm* **3, 100057.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Hashim, A. A., Kadir, A. A., Ibrahim, M. H., Halim, S., Sarani, N. A., Hassan, M. I. H. *et al.* (2021). Overview on food waste management and composting practice in Malaysia. *AIP Conference Proceedings* **2339, 020181.**

Hassan, S. H. A., Zohri, A. N. and Kassim, R. M. F. (2019). Electricity generation from sugarcane molasses using microbial fuel cell technologies. *Energy* **178, 538-543.**

Idris, M. O., Kim, H. C., Yaqoob, A. A. and Ibrahim, M. N. M. (2022). Exploring the effectiveness of microbial fuel cell for the degradation of organic pollutants coupled with bio-energy generation. *Sustainable Energy Technologies and Assessments* **52, 102183.**

Iriarte, D., Andrada, H., Ochoa, S. A. M., Silva, O. F., Chávez, F. V. and Carreras, A. (2022). Effect of acid treatment on the physico-chemical properties of Nafion 117 membrane. *International Journal of Hydrogen Energy* **47(49), 21253-21260.**

Ishii, S., Logan, B. E. and Sekiguchi, Y. (2012). Enhanced electrode-reducing rate during the enrichment process in an air-cathode microbial fuel cell. *Applied Microbiology and Biotechnology* **94(4), 1087-1094.**

Islam, P. (2020). Creating bacterial glycerol stocks for long-term storage. dx.doi.org/10.17504/protocols.io.bf5qjq5w

Jenol, M. A., Ibrahim, M. F., Bahrin, E. K., Kim, S. W. and Abd-Aziz, S. (2019). Direct bioelectricity generation from sago hampas by *Clostridium beijerinckii* SR1 using microbial fuel cell. *Molecules* **24, 2397.**

Koffi, N. J. and Okabe, S. (2020). High voltage generation from wastewater by microbial fuel cells equipped with a newly designed low voltage booster multiplier (LVBM). *Scientific Reports* **10, 18985.**

Krithika, T., Kavitha, R., Dinesh, M. and Angayarkanni, J. (2021). Assessment of ligninolytic bacterial consortium for the degradation of azo dye with electricity generation in a dual-chambered microbial fuel cell. *Environmental Challenges* **4, 100093.**

Logan, B. E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S*. et al.* (2006). Microbial fuel cells: Methodology and technology. *Environmental Science and Technology* **40(17), 5181-5192.**

Logan, B. E., Rossi, R., Ragab, A. and Saikaly, P. E. (2019). Electroactive microorganisms in bioelectrochemical systems. *Nature Reviews Microbiology* **17(5), 307-319.**

Majumder, D., Maity, J. P., Tseng, M. J., Nimje, V. R., Chen, H. R., Chen, C. C. *et al.* (2014). Electricity generation and wastewater treatment of oil refinery in microbial fuel cells using *Pseudomonas putida*. *International Journal of Molecular Sciences* **15(9), 16772-16786.**

Makhtar, M. M. Z. and Tajarudin, H. A. (2020). Electricity generation using membrane-less microbial fuel cell powered by sludge supplemented with lignocellulosic waste. *International Journal of Energy Research* **44(4), 3260-3265.**

Miran, W., Nawaz, M., Jang, J. and Lee, D. S. (2016). Conversion of orange peel waste biomass to bioelectricity using a mediator-less microbial fuel cell. *Science of the Total Environment* **547, 197-205.**

Pei, P., Jia, X., Xu, H., Li, P., Wu, Z., Li, Y. *et al.* (2018). The recovery mechanism of proton exchange membrane fuel cell in micro-current operation. *Applied Energy* **226, 1-9.**

Phooi, C. L., Azman, E. A., Ismail, R., Shah, J. A. and Koay, E. S. R. (2022). Food waste behaviour and awareness of Malaysian. *Scientifica* **2022, Article ID 6729248.**

Priya, A. D. and Setty, Y. P. (2019). Cashew apple juice as substrate for microbial fuel cell. *Fuel* **246, 75-78.**

Rojas-Flores, S., Benites, S. M., De La Cruz-Noriega, M., Cabanillas-Chirinos, L., Valdiviezo-Dominguez, F., Álvarez, M. A. Q. *et al.* (2021a). Bioelectricity production from blueberry waste. *Processes* **9(8), 1301.**

Rojas-Flores, S., Cabanillas-Chirinos, L., Nazario-Naveda, R., Gallozzo-Cardenas, M., Diaz, F., Delfin-Narciso, D. *et al.* (2023). Use of tangerine waste as fuel for the generation of electric current. *Sustainability* **15(4), 3559.**

Rojas-Flores, S., Pérez-Delgado, O., Nazario-Naveda, R., Rojales-Alfaro, H., Benites, S. M., De La Cruz-Noriega, M. *et al.* (2021b). Potential use of papaya waste as a fuel for bioelectricity generation. *Processes* **9(10), 1799.**

Saleh, A. F., Kamarudin, E., Yaacob, A. B., Yussof, A. W. and Abdullah, M. A. (2012). Optimization of biomethane production by anaerobic digestion of palm oil mill effluent using response surface methodology. *Asia-Pacific Journal of Chemical Engineering* **7(3), 353-360.**

Sulong, M. R., Hamid, H., Sivam, A., Abdullah, H. and Wee, L. L. (2021). Utilization of banana peel-wastes for the production of low-cost and Shariah-compliant amylolytic enzyme. *In*: Enhancing Halal Sustainability. Shariff, N. N. M., Lateh, N., Zarmani, N. F., Hamidi, Z. S., Aghwan, Z. A. A. Mahalle, N. B. D. H. *et al.* (eds.). Springer, Singapore. **pp. 249-258.**

Tan, B. C. (2022). Can banana be a success story for Malaysia? *Journal of Agribusiness Marketing* **9(1), 13-22.**

Thapa, B. S. and Chandra, T. (2019). *Kluyvera georgiana* MCC 3673: A novel electrogen enriched in microbial fuel cell fed with oilseed cake. *Current Microbiology* **76(5), 650-657.**

Verma, M. and Mishra, V. (2022). Bioelectricity generation by microbial degradation of banana peel waste biomass in a dual-chamber S. cerevisiae-based microbial fuel cell. *Biomass and Bioenergy* **168, 106677.**

William, P., Wen, E. T. X. and Jenevieve, H. J. Y. (2019). Microbial fuel cells: Food waste as a sugar source. *In*: Guo, H., Ren, H. and Bandla, A. (eds.). *IRC-SET 2018: Proceedings of the 4th IRC Conference on Science, Engineering and Technology*. Springer, Singapore. **pp. 143-151.**

Yaqoob, A. A., Bin Abu Bakar, M. A., Kim, H. C., Ahmad, A., Alshammari, M. B. and Yaakop, A. S. (2022). Oxidation of food waste as an organic substrate in a single chamber microbial fuel cell to remove the pollutant with energy generation. *Sustainable Energy Technologies and Assessments* **52, 102282.**

Yaqoob, A. A., Ibrahim, M. N. M. and Guerrero-Barajas, C. (2021). Modern trend of anodes in microbial fuel cells (MFCs): An overview. *Environmental Technology and Innovation* **23, 101579.**

Zahrim, A. Y., Asis, T., Hashim, M. A., Al-Mizi, T. M. T. M. A. and Ravindra, P. (2015). A review on the empty fruit bunch composting: Life cycle analysis and the effect of amendment(s). *In*: Advances in Bioprocess Technology. Ravindra, P. (ed.). Springer, Cham. **pp. 3-15.**

Zapata, A. and Ramirez-Arcos, S. (2015). A comparative study of McFarland turbidity standards and the Densimat photometer to determine bacterial cell density. *Current Microbiology* **70, 907-909.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538