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Local Fruit Waste Driven Benthic Microbial Fuel Cell: A Sustainable Approach to Toxic Metal Removal and Bioelectricity Generation

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Abstract:

The present work focused on the utilization of three local wastes i.e., rambutan (*nephelium lappaceum*), langsung (*lansium parasiticum*) and mango (*mangifera indica*) wastes as organic substrates in benthic microbial fuel cell (BMFC) to reduce the cadmium and lead concentrations from synthetic wastewater. Out of the three wastes, the mango waste promoted a maximum current density (87.71 mA/m²) along with 78 % and 80 % removal efficiencies for Cd²⁺ and Pb²⁺, respectively. The bacterial identification proved that *Klebsiella pneumoniae*, *Enterobacter*, and *Citrobacter* were responsible for metals removals and energy generation. Lastly, the BMFC mechanism, challenges and future recommendations are enclosed.

Keywords: Benthic microbial fuel cell; Wastewater; Heavy Metals; Bioenergy; Bacteria identification.

1. Introduction:

Modern human society faces critical challenges arising from the crisis of renewable energy and clean water supply around the world. This problem arises mainly due to population growth and industrial activities in which several other factors are also involved (Yaqoob et al. 2020c). Despite all the developments, there are still issues that need to be solved, which prompts researchers to find economical, environmentally friendly, fast, and reliable methods to produce bioenergy and control water pollution. In the modern world, high demand for energy is increasing due to the use of non -renewable energy at the industrial level which is causing a decline in natural fossil fuels. Rapid industrial growth is primarily responsible for the energy crisis, moreover, the practice of solid waste burning has caused serious damage to water resources. Water pollution has a direct impact on human survival. Currently, the world faces the presence of heavy metals in water sources that can cause serious health problems. Therefore, there is a critical challenge to find mechanisms to control water pollution and at the same time produce bioenergy. To address these issues, several studies suggested that the microorganisms can act as catalysts (i.e., *Geobacter* sp., *E. Coli*, *Pseudomonas* sp. etc.) to produce bioenergy and minimize the heavy metal concentration from water resources (Fadzli et al. 2021a, Logan et al. 2006, Yaqoob et al. 2020b). Microbial fuel cells (MFC) has been introduced as an environmental biotechnology practice that has received a lot of attention from researchers. MFC is an environmentally friendly system and a safe approach to wastewater treatment, but it requires a synthetic organic substrate, which serves as a fuel to promote effective oxidation by microorganisms (Asim et al. 2021, Logan et al. 2006). This is especially true in cases where highly electrogenic microorganism strains (such as *Geobacter* sp.) are involved in the system (Li et al. 2018, Logan 2009).

Recently, an updated version of MFC has been introduced to remediation practices to utilize naturally derived organic substrates to replace synthetic organic substrates. This version of the MFC is a benthic microbial fuel cell (BMFC), which operates based on the same concept as the MFC (Nielsen et al. 2007, Yaqoob et al. 2021b). The main difference between BMFC and MFC is the use of organic substrates derived from the reduction of organic matter that occurs naturally in the environment. BMFC does not require the addition of external fuels as bacterial feed during its process (Umar et al. 2021). BMFC uses natural waste as an organic substrate in the anodic space to promote bacterial growth for the oxidation process and this waste also serves as a pseudo-membrane to allow proton flow in BMFC. Anaerobic bacteria function in the anodic chamber of the BMFC, whereas the cathode is exposed to aerated conditions (Umar et al. 2020). In several studies reported the use of organic substrates derived from waste in MFC, however little work needs to be done for BMFC cases, especially those related to heavy metal removal (Joiner et al. 2020, Pushkar & Mungray 2020, Tavakolian et al. 2020). According to Vilarino Franco and Quarrington (2017), about 1.3 billion tons of food is wasted each year worldwide (Vilariño et al. 2017). The BMFC system is an efficient approach to minimize waste from the environment and produce bioenergy by using waste materials.

In the present work, some local fruit wastes such as rambutan (*nephelium lappaceum*), langsat (*lansium parasiticum*), and mango (*mangifera indica*) were utilized as organic substrates in BMFC to promote bacterial activity during the generation of electrons and protons. Rambutan, langsat, and mango are suitable to be grown throughout Malaysia and several Southeast Asian nations such as Indonesia and Thailand. These fruits will produce waste in the form of skins and seeds. The targeted wastes are rich sources of organic matter (carbohydrates and glucose) as having been reported in the literature. To our knowledge, at present, there is no work on the use of these

wastes in BMFC even though these are very rich organic resources and is available in relatively large quantities in Malaysia. In addition, our work demonstrates that BMFC is capable of being a generator of bioenergy, which is derived from the oxidation of natural wastes in the anodic space in a process combined with the removal of heavy metals from synthetic wastewater. This work is limited to the removal of cadmium and lead ions. Furthermore, this study also focuses on the optimization of parameters (pH and temperature) to improve BMFC performance.

2. MATERIALS AND METHODS

2.1. Sample preparation and inoculum source

Targeted wastes such as rambutan, langsat, and mango waste were received from the local market of Penang, Malaysia. The waste is gently washed to remove impurities and cut into small pieces. These small pieces of garbage are stored at room temperature. Similarly, synthetic wastewater was prepared by collecting wastewater from a pond in Gelugor, Penang, Malaysia, which was further supplemented with a solution of lead nitrate (Sigma-Aldrich) and 50-ppm cadmium nitrate (Sigma-Aldrich). The physiochemical properties of the wastewater collected before and after treatment are shown in Table 1. The treated wastewater is known as synthetic wastewater in the present study. Electrical conductivity (ECM) (measured by an Alpha-800 conductivity meter), liquid temperature (measured with a GH thermometer, ZEAL LTD, England), and pH (measured with an EUTECH-700 USA pH meter), were used to determine conductivity, temperature, and wastewater pH.

Table 1: Physicochemical properties of the collected wastewater before and after metal supplementation

Parameters	Untreated wastewater	Treated wastewater
Color	Light black	Light black
Odor	unfriendly smell	unfriendly smell

pH	7.04	6.50
Temperature	25-30°C	25-30°C
Electrical conductivity	59 μ S/cm	80 μ S/cm
Cd ²⁺	0 ppm	50 ppm
Pb ²⁺	0 ppm	50 ppm

2.2. Benthic microbial fuel cells construction and operation

A double chamber BMFC was constructed by joining two commercial cylindrical glasses. The dimensions for the constructed BMFC chambers (anode and cathode) were 10 cm in length and 10 cm in diameter. The total volume of one chamber was 500 mL and the working volume was 400 mL. The anodic chamber was filled with synthetic wastewater (400 mL) along with waste material (each waste was 150 g) as an organic substrate for bacteria. The supplemented metal ions were present in synthetic wastewater. The mixed culture of bacterial species was present as the inoculum which oxidizes the organic substrate. The graphite rod (GF series, FUDA 2B Lead, NY, USA) with dimensions of 10 cm $\times$ 2 cm (h $\times$ r) served as anode and cathode electrodes in both chambers. The carbon-based material is an efficient source for remediation and energy applications [16]. The distance between the two electrodes was 7 cm. The BMFC anodic chamber was closed to maintain an anaerobic atmosphere, whereas the cathodic chamber was filled with aerated tap water. To maintain an airy environment in the cathodic space, an aquarium pump was used to supply oxygen. These features make BMFC more prominent than MFC, which saves time and effort due to the simultaneous use of natural waste and energy generation. A medium-size copper wire was used throughout the operation to provide external loads (560 Ω). Three types of local fruit wastes were used as organic substrates in the anodic chamber. The performance of BMFC

with each of these residues was labeled as: BMFC-I (rambutan waste), BMFC-II (langsar waste), and BMFC-III (mango waste). All BMFC experiments were performed in a similar manner as described above. All BMFC experiments were performed at room temperature under continuous operation for 45 days. To evaluate and optimize the influence of pH and temperature on energy generation and metal removal efficiency, pH was assessed in the range of 3–10 and temperature in the range of 15–35 °C in each case. The lab scale design of the BMFC is shown in Fig. 1.

Fig. 1: Graphic presentation of the BMFC system.

2.3. Electrochemical measurements

The cell potential voltage (V) was recorded using a digital multimeter (UNI-T, Model UT120A, China). The voltage of each BMFC is measured once a day and Ohm's law is followed to calculate the current density/power density of each cell. The following equations were used to calculate the force density (PD) and current density (CD), as well as the current and internal resistance of the cell (Chen et al. 2017, Salvin et al. 2015).

$$V=IR \text{ -----(1)}$$

$$PD = \frac{V^2}{RA} \text{ -----(2)}$$

$$CD = \frac{I}{A} \text{ -----(3)}$$

$$r = \left[\frac{E - V}{I} \right] R \text{-----(4)}$$

where,

V=potential voltage; r= internal resistance; I= current; R= external resistance; A= surface area; E= Electromotive force (Emf). The value of E can be determined by employing the open-circuit voltage (OCV). Further, the polarization curve (V–I) was studied by following the ‘Rext-variation’ system which was defined by Logan et al. (Logan et al. 2006). An adjustable resistor box was attached to the system to regulate the external resistance of the cell from 10 kΩ to 100 Ω (open-circuit t-short-circuit). The value of each resistance was measured after the establishment of the pseudo-steady state. Usually, a 10-20 min variation was required during measurements. Therefore, the polarization curve was calculated, and it illustrates the relationship power (P) versus I. Similarly, the internal resistance calculation was carried out by following Equation 4.

Furthermore, cyclic Voltammetry (CV) was carried out to study the redox reaction status during the operation of BMFC-I, BMFC-II, and BMFC-III. CV measurements were carried out at different time intervals i.e., on the 15th day, 30th day, and 45th day. The CV measurement protocol was as follows: 20 mV/s rate in a -0.8 to +0.8 V of potential range. The glassy carbon, Ag/AgCl, and Pt wire were used during the measurements as the working electrode, counter electrode, and reference electrode, respectively.

In addition, to complete the CV evaluation, the specific capacitance (Cp, (F/ g)) was also calculated. Equation 5 is used to calculate the value of Cp.

$$C_p = \frac{A}{2mk(V_2 - V_1)} \text{-----(5)}$$

where, (V2 – V1)= potential range of CV; m = loaded sample (g), A = area of CV curves (AV), k = scan rate of CV in mV/s.

2.4. Metal removal analyses and biological studies

The metal analysis was carried out through an atomic absorption spectrometer (PerkinElmer AAnalyst 400). Each cell BMFC-I, BMFC-II, and BMFC-III was filled with synthetic wastewater and targeted wastes which contained a 50-ppm solution of Cd^{+2} and Pb^{+2} . A sample of 1.5 mL of the synthetic wastewater was taken every 10 days according to the planned schedule. Equation 6 was used to calculate the removal efficiency of the metals from the cells.

$$\text{R.E \%} = \frac{\text{TM}_i - \text{TM}_f}{\text{TM}_i} \times 100 \text{ -----(6)}$$

where, R.E.= removal efficiency %; TM_i = total initial toxic metal concentration; TM_f = total final toxic metal concentration. Scanning electron microscopy (SEM) was used to study the biofilm morphology. Biofilm is a conglomerate of bacteria formed on the surface of the anode, which is responsible for metal removal. The SEM images of the biofilm showed the presence of bacteria on the surface of the anode which generally is involved in the metal removal mechanism as has been already discussed in previous studies (Chen et al. 2011, Rajeswari et al. 2016, Tang et al. 2014). To identify the bacterial species involved in the process occurring at the anode surface of each BMFC (BMFC-I, BMFC-II, and BMFC-III), isolation and identification of bacteria were performed. At the end of the operation, the anode was treated with sterilized water before the biofilm was carefully pruned using a sterilized knife. The collected biofilm was further treated through a serial dilution process (10^{-1} to 10^{-9}) for bacterial growth on nutrient agar plates until the growth of single colonies was observed. The pure colonies were further assessed through colony polymerase chain reaction (PCR). The PCR was carried out to amplify the DNA extracted from the isolated bacteria. The 1492R reverse primer and 27F forward primer were used for the amplification. The 16S rRNA sequences were submitted to the GenBank under the accession numbers listed in Table 5.

3. Results and Discussion

3.1. Energy output from natural wastes without any substrate additional in BMFC

The observed potential voltage outputs of the BMFC-I, BMFC-II, and BMFC-III systems made with a fresh inoculum source and an external load of 560 Ω , are shown in in Figure 2a.

According to the curves of the three BMFC systems observed, a major difference was observed in the voltage generation on the first day in the presence of an external load of 560 Ω . All BMFC operations were carried out for 45 days until the voltage dropped to the lower limit. Open-circuit voltages were also observed at the beginning of the reaction without external resistance, these values being 300 mV (BMFC-I), 270 mV (BMFC-II), and 490 mV (BMFC-III). The power outputs of BMFC-I, BMFC-II, and BMFC-III on the first day were 0.017 mA, 0.001 mA, and 0.026 mA, respectively. The current generation difference is quite high between BMFC-I and BMFC-II. Similarly, the highest output current difference was 0.025 mA observed in BMFC-II and BMFC-III. BMFC-I showed the highest voltage on the 20th day (170 mV), BMFC-II showed the highest voltage on the 24th day (100 mV) and BMFC-III showed 180 mV on the 18th day. According to the observed potential, the highest calculated current densities were 39.94 mA/m² (BMFC-I), 23.49 mA/m² (BMFC-II), and 42.29 mA/m² (BMFC-III). Further, after reaching the highest point, the voltage starts to drop sharply to reach the minimum level, this is true for BMFC-I, and BMFC-II, which showed a minimum of 0 mV on the 40th day and BMFC-III shows 1 mV on the 42nd day. The BMFC voltage decreases sharply after a certain point because the level of the organic substrate, which was the food source of bacteria was decreasing in the anodic space. The highest voltage point also indicated the maximum removal of metal ions (Pb²⁺ and Cd²⁺ ions) from the wastewater. Overall, the presence of mango waste (skin and seeds) in BMFC-III was associated with the highest voltage and stability during operation compared to other cells (BMFC-I and

BMFC-II). The results indicated that the level of glucose in the waste was an important factor in maintaining bacterial growth and electron generation during bacterial respiration.

3.2. Electrochemical measurement

To analyze the effect of PD vs V, various external resistances were tested (150 Ω - 7 k Ω). High external resistances such as 7 k Ω or 6 k Ω indicated fast potential stability, but the CD and PD trends lead to lower values compared to low external resistances such as 150, 200 or 560 Ω . Similarly, voltage instability was lower at higher external resistances (2–7 k Ω) than at lower external resistances (150, 300, 560 Ω). High voltage instability at lower external resistances due to the flow of electrons towards the anode was effective because there was little resistance interfering with the flow. Similarly, high resistance prevents the flow of electrons during the transformation of organic matter toward the cathode, which resulted in low voltage stabilization. In the present work, all BMFC were connected to a resistance box by varying the external resistance from 150 Ω to 7 k Ω . The voltage values from a high resistance (7 k Ω) to a lower resistance (150 Ω) were of 290 mV to 42 mV (BMFC- I), 220 mV to 45 mV (BMFC- II), and 400 mV to 100 mV (BMFC- III) as shown in Fig. 2 (a-d). The voltage was shifting from a high to a low value as the variation of the resistances shifted from high to low (7 K Ω to 15 Ω). However, the highest CD recorded was of 36.84 mA/m² (for BMFC- I), 39.96 mA/m² (for BMFC- II), and 87.71 mA/m² (for BMFC- III) at lower resistances. Similarly, PD values were recorded as 0.30 mW/m² (BMFC-I), 0.22 mW/m² (BMFC-II) and 0.71 mW/m² (BMFC-III) at external resistances of 560 Ω and 450 Ω . Similarly, the internal resistance of the cell was also important for the release of electrons from the anode to the cathode. Equation 4 was used to calculate the internal resistance of the cell for 45 days of BMFC operation. BMFC- I, BMFC- II, and BMFC- III showed internal resistance values of 560,

728 and 544 Ω , respectively from which the PD/CD ratio can be obtained with this internal resistance. Usually, an external oxygen supply also helps stabilize the voltage at high resistance.

A similar trend was also observed by Reimers et al. (2006), they recommended the use of BMFC in naturally rich organic substrates to improve BMFC performance. Nielsen et al. (2007) also recommended a new design for BMFC with natural waste as the organic substrate in the anodic space to achieve the highest pollution elimination and energy generation.

Fig. 2: (a) Observed voltages for BMFC- I, BMFC- II and BMFC-III (b) polarization curves of BMFC- I (c) polarization curves of BMFC- II (d) polarization curves of BMFC- III with external load variations from 150 Ω to 7 k Ω .

CV analysis showed the bacterial biomass of the electrodes at different time intervals to assess the rate of oxidation and reduction during operation. Each BMFC was analyzed separately at different time intervals to observe oxidation and reduction rates. BMFC-I showed maximum currents in the forward scan at 1.0×10^{-5} μA (on the 15th day), 6.4×10^{-5} μA (on the 30th day), and 8.0×10^{-5} μA (on the 45th day), while the other part of the scan showed -0.0×10^{-5} μA (on the 15th day), $-0.5 \times$

$10^{-5} \mu\text{A}$ (on the 30th day), and $-1.1 \times 10^{-5} \mu\text{A}$ (on the 45th day). BMFC-II showed maximum currents in the forward scan at $1.1 \times 10^{-5} \mu\text{A}$ (Day 15th), $4.3 \times 10^{-5} \mu\text{A}$ (Day 30th), and $5.4 \times 10^{-5} \mu\text{A}$ (Day 45th), whereas for the reverse scan it showed $-0.2 \times 10^{-5} \mu\text{A}$ (Day 15th), $-0.8 \times 10^{-5} \mu\text{A}$ (Day 30th), and $-1.0 \times 10^{-5} \mu\text{A}$ (Day 45th). Similarly, BMFC-III showed in the forward scan of $0.9 \times 10^{-5} \mu\text{A}$ (Day 15th), $1.5 \times 10^{-5} \mu\text{A}$ (Day 30th), and $3.0 \times 10^{-5} \mu\text{A}$ (Day 45th), whereas in the reverse scan it showed $-0.2 \times 10^{-5} \mu\text{A}$ (Day 15th), $-1.2 \times 10^{-5} \mu\text{A}$ (Day 30th), and $-5.5 \times 10^{-5} \mu\text{A}$ (Day 45th). According to the results, the BMFC-I, BMFC-II, and BMFC-III showed the maximum current at forward and reverse scans rate on the 45th day. The forward and reverse scans rate are indication of the rates of reduction and oxidation during the BMFC operation. The results showed that the rate of reduction and oxidation gradually increased and achieved a maximum on the 45th day. It is also an indication that the operation in the cell has been completed. Afterward, the metal reduction, as well as voltage generation, was gradually decreasing due to the completion of the bacterial life cycle or completion of the operation of the cell. With respect to oxidation (E_{oxid}) and reduction (E_{red}) for each BMFC operation, the results are as follows: the BMFC-I showed $E_{\text{oxid}} = 0.8\text{V}$ and $E_{\text{red}} = -0.8\text{V}$ on the 15th day; $E_{\text{oxid}} 0.8\text{V}$ and -0.79V E_{red} on day 30th and $E_{\text{oxid}} 0.8\text{V}$ and -0.78V E_{red} on day 45th. The BMFC-II showed $E_{\text{oxid}} 0.8\text{V}$ and -0.8V E_{red} on day 15th; $E_{\text{oxid}} 0.8\text{V}$ and -0.79V E_{red} on day 30th and $E_{\text{oxid}} 0.8\text{V}$ and -0.77V E_{red} on day 45th. The BMFC-III showed $E_{\text{oxid}} 0.8\text{V}$ and -0.78V E_{red} on day 15th; $E_{\text{oxid}} 0.8\text{V}$ and -0.76V E_{red} on day 30th and $E_{\text{oxid}} 0.8\text{V}$ and -0.75V E_{red} on day 45th. Each BMFC operation showed a higher rate of oxidation at the beginning as compared to the later days. The reduction rate also decreased at the end of the reaction, which indicates the completion of the operation. In literature, various studies reported this trend (Xu et al. 2017, Yaqoob et al. 2021a). For example, Yaqoob et al. (2020a) demonstrated that the lignin-derived GO anodes in the presence of oil palm trunk sap as organic substrate can promote the removal of lead ions from

synthetic wastewater. These authors followed a similar procedure to elaborate and support their results.

In addition, other parameters were considered to evaluate the trend of biofilm formation at the BMFC anode during operation. The CV curve allows the determination of C_s . The calculated C_s showed that the biofilm had excellent strength throughout the operation. High C_s values indicated good biofilm stability and formation. In this study, each BMFC system showed stable biofilm activity as shown in Table 2. Several studies have also found similar observations to explain and indicate strong biofilm formation (Daud et al. 2021, Fadzli et al. 2021b, Yaqoob et al. 2020a).

Table 2: Calculated specific capacitance, C_s , data from CV curves during the operation of each BMFC.

Measurement		Specific Capacitance (F/g)		
time	interval	BMFC-I	BMFC-II	BMFC-III
(days)				
15 th		0.00004	0.00006	0.00043
30 th		0.00008	0.00008	0.00052
45 th		0.00011	0.00011	0.00059

Fig. 3: CV behavior of BMFC-I, BMFC-II and BMFC-III at different time intervals during the operation.

3.3. Effect of pH on energy generation in the presence of natural waste in BMFC

According to the observations, Fig. 4a shows a direct relationship between the pH of the solution and voltage generation. The BMFC were operated at the different ranges of pH solution in the anodic chamber. The pH of the solutions (BMFC- I, BMFC-II, BMFC-III) was adjusted by using either 1 M NaOH or 1 M HCl solution. Solution pH optimization is required to obtain a high PD/CD ratio. This is a very critical and important point in the experimental work. In the present study, the pH values considered for the treated solutions in the cells were 3, 4, 5, 6, 7, 8, and 10. Measurements of each pH range were recorded after 10 days of operation. The results confirmed that the natural pH is the optimal condition to reach the maximum voltage. At pH 7, BMFC-I, BMFC-II, and BMFC-III showed potential values of 120 mV, 35 mV, and 124 mV, respectively. At pH 3, BMFC-I, BMFC- II, and BMFC- III has the potential value of 24 mV, 5 mV, and 26 mV, respectively. Similarly, at pH 10, the value was 20 mV, 9 mV, and 44 mV for BMFC-I, BMFC-II, and BMFC- III, respectively. The conclusion is that a highly acidic or basic condition disturbs the bacterial growth and damages the biofilm on the anode, which led to the decrease of the potential.

He et al. (2008) studied the operation of MFC in the pH range of 5–6 and reported low PD. They concluded that natural pH is a critical condition for producing sufficient voltage. Another study was carried out by Huang et al. (2012) observed the effect of pH on power density, where they concluded that at pH 5.2, the voltage was low due to the generation of acidic by-products, whereas treatments at natural pH presented a clear increment in voltage generation. Similarly, Din et al. (2020) also concluded that the neutral pH is the optimum condition in MFC when using waste material (potatoes waste) as organic substrate. Both extremes in pH value of the media in the cells, either too acidic or too alkaline, are not suitable for bacterial growth at which organic waste is used as substrate in BMFC.

3.4. Effect of the temperature on energy generation in the presence of natural waste in BMFC

The BMFC were operated at a different temperature range from 15 °C to 35 °C. The range is slightly higher than room temperature, or slightly lower than room temperature. The objective was to optimize the temperature effect on energy generation in BMFC. Our results indicated that 25 °C is suitable for BMFC operation to achieve the maximum energy output (Fig. 4b). BMFC-I, BMFC- II, and BMFC- II exhibited a potential value of 45 mV, 39 mV, and 49 mV, respectively. All BMFC reactions were observed at pH 7 with 560 Ω external resistances. The measurements were recorded after 24 h of operation. The temperature range (15-20 °C) that was lower than room temperature led to a very low voltage potential due to the harsh conditions for bacterial growth. All exoelectrogenic bacteria require an optimum condition for growth that also includes a favorable temperature. Another aspect observed is that below 20 °C there is still a voltage generated, for example, 5-9 mV (BMFC- I), 2-5 mV (BMFC- II), and 5-12 mV (BMFC- III) but at temperatures of 15 °C and below the survival level of the bacteria is doubtful.

Fig. 4: (a) Effect of pH (b) effect of temperature on energy generation during BMFC operation.

3.5. Effect of organic substrate in BMFC

The organic substrate is the energy source required for the bacterial growth and their respiration process during the BMFC operation, which leads to the generation of electrons and protons and concurrently pollutant remediation. The organic substrate is one of the most important aspects of all the bioelectrochemical cells because bacteria require fuel for respiration during the oxidation process (Barbosa et al. 2017). To our knowledge, very rarely efforts have been made to use natural waste as an organic substrate in BMFC applications (Fadzli et al. 2021a). Several types of organic substrates such as glucose, protein, carbohydrate, sucrose, fructose, cellulose vegetable waste, and biomass waste have been used in MFC studies (Gezginci & Uysal 2016, Meshram & Jadhav 2017, Pant et al. 2010). These natural wastes can be taken as an organic substrate, which is to minimize operating costs if the system is implemented on a large scale.

Salvin et al. (2015) studied mangroves as an organic substrate in BMFC to produce bioenergy and they achieved significant results while controlling operating costs. Unfortunately, there is still a lack of reports reflecting efforts to use natural organic waste in BMFC that allow us to compare our results. Therefore, we have considered reports in which some natural wastes have

been used as organic substrates in MFC to discuss the importance of our work. In this study, the BMFC operation was not given an additional substrate except the waste materials placed at the beginning of the operation. Table 3 shows a comparison of works in which waste materials have been used in several MFC systems.

Table 3: Comparison summary of previous reports on waste material and CD outcomes obtained with different organic substrates in MFC.

Type of fuel cell	Substrate	Inoculum source	Current density (mA/cm ²)	Reference
DMFC	Landfill leachate	Leachate and sludge	0.0004	(Greenman et al. 2009)
DMFC	Macroalgae, Ulva lactuca	Mixed culture	0.025	(Velasquez-Orta et al. 2009)
DMFC	Malt extract, yeast extract and glucose	Pure culture of <i>E. cloacae</i>	0.067	(Mohan et al. 2008)
DMFC	Microcrystalline cellulose	-	0.02	(Rismani-Yazdi et al. 2008)
SMFC	Brewery wastewater	Full strength brewery wastewater	0.2	(Feng et al. 2008)
SMFC	Beer brewery wastewater	Anaerobic mixed consortia	0.18	(Wen et al. 2009)

DMFC	Chocolate industry wastewater	Activated sludge	0.302	(Patil et al. 2009)
BMFC	Rambutan waste wastewater	Synthetic	0.0036	Present work
BMFC	Langsat waste wastewater	Synthetic	0.029	Present work
BMFC	Mango waste wastewater	Synthetic	0.0087	Present work

DMFC= Double chamber microbial fuel cell; SMFC= Single chamber microbial fuel cell; BMFC= Benthic microbial fuel cell

3.6. Removal of toxic metals from synthetic wastewater through BMFC

The potential in the BMFC was generated through electrons flow, these electrons were released from the anode because of the oxidation and reduction of metals in the anodic chamber. The electrons were transferred to the cathode electrode through the external resistance and therefore, the removal of the metal may be related in a positive way to the energy generation (Serrà et al. 2021) . The wastewater that was used in the present work was spiked with Cd^{2+} and Pb^{2+} . The detected concentrations of metal during and at the end of the operation are summarized in Table 4. At the beginning of the BMFC operation, the concentration of metals (50 ppm) was considered as the initial concentration and after the operation of the cells, the final metal concentration of the metals allowed the calculation of the metal removal. According to Table 4, BMFC-III showed an excellent removal efficiency of Cd^{2+} and Pb^{2+} . The highest removal efficiency of Cd^{2+} and Pb^{2+} was 78 % and 80 %, respectively after 40-45 days of operation. Similarly, BMFC-II showed the lowest performance in terms of removal of Cd^{2+} and Pb^{2+} than BMFC-III. All the BMFC were

performed with the same parameters, usually, a low redox potential is required for metal removal. Out of the three systems, the BMFC-III presented the best results because the organic substrate (mango waste) provides enough energy to bacteria for survival in a highly toxic environment, which efficiently promotes the removal of metals. It was observed that over time, the performance of the cell (catalyzed by the bacteria) was affected due to the consumption of substrate. The constant supply of organic substrates as fuel for bacteria remains a challenge in BMFC. The short-term availability of fuel cells limits the performance of BMFC for its use on an industrial scale.

Table 4: Removal trend of targeted metals via BMFC in the presence of different organic substrates.

MFC reactors	Organic substrate	Incubation media	Initial concentration (ppm)	Target pollutant	Operational time (Days)	Removal efficiency (%) of Cd^{2+}	Removal efficiency (%) of Pb^{2+}
BMF C-I	Rambutan	Synthetic wastewater	50	Cd^{2+} & Pb^{2+}	0	0	0
					10	13	16
					20	28	26
					30	40	39
					40	49	50
					45	60	68
BMFC-II	Langsat	Synthetic wastewater	50	Cd^{2+} & Pb^{2+}	0	0	0
					10	5	6
					20	21	22

						30	32	35
						40	58	60
						45	69	71
BMFC-	Mango	Synthetic	50	Cd ²⁺	&	0	0	0
III		wastewater		Pb ²⁺		10	16	18
						20	31	29
						30	42	44
						40	65	69
						45	78	80

3.7.Effect of pH on metal removal from synthetic wastewater

The pH effect on the removal efficiency of Cd²⁺ and Pb²⁺ were clearly observed, and it is shown in Fig. 5a. In all BMFC setups, the pH was adjusted by using 1M NaOH and 1M HCl solutions and the removal efficiency at each pH was recorded after 10 days of operation. The results showed that as pH increases from 3 to 10, a clear removal percentage (%) was observed. Out of the three systems, the BMFC-III presented quite significant removal efficiency within 10 days for Cd²⁺ and Pb²⁺, i.e., 20% and 22%, respectively at pH 7. These were the highest percentages of removal observed. BMFC-I, BMFC-II also showed the highest removal efficiency at pH 7 compared to other pH ranges. On the contrary, pH 3 was a highly acidic condition and pH 10 was a highly alkaline condition, where both conditions were not favorable for bacterial growth. Neutral pH increases the high removal efficiency due to the diffusion of H⁺ ions from the cathodic

to the anodic space as the pH decreases. The acidic anodic environment has a negative impact on bacterial activity, especially inhibiting growth as well as oxidation/reduction processes.

Effect of temperature on metals removal from synthetic wastewater

BMFC- I, BMFC- II and BMFC- III were operated at different temperatures of 15 ° C to 35 ° C for the removal of Cd^{2+} and Pb^{2+} (Fig. 5b). Out of the three, BMFC- III showed the highest removal efficiencies of 15.8% and 18.2% for Cd^{2+} and Pb^{2+} , respectively, at room temperature. In all the BMFC, as temperature increased from 15°C, the removal efficiency increased slowly. All the BMFC showed the highest removal efficiency at 25°C and the lowest removal efficiency at 15°C. The temperatures of 15°C and 35°C are not favorable for bacteria survival. Most of the bacteria start the decay phase at temperatures above 35°C or below 20°C. Some bacteria can survive above 35°C under optimized and controlled conditions (Lechevallier et al. 1988). At lower and higher temperature ranges, the rate of toxic metal removal becomes low. At room temperature, the removal rate increases due to a decrease in ohmic resistance, an increase in metabolic pathways, and greater water conductivity. The temperature and ohmic resistance, both are inversely proportional. As ohmic resistance increase, the temperature will be lower and vice versa due to conductive stability of metal ions. However, the ohmic resistance variation is not only restricted to fluctuations in the temperature range. Out of all the systems, the BMFC- III showed the maximum removal rate because bacteria tended to thrive in this system due to a sufficient supply of energy in form of organic substrate. The other systems (BMFC-I and BMFC- II), however, failed to supply sufficient energy for bacterial activity during their operations.

Fig. 5: (a) Effect of pH (b) effect of temperature on the removal of metals from synthetic wastewater

3.8. Biofilm analyses and operational mechanism of BMFC

The biofilm formed on the surface of the anode is well known as a niche of microbes. The presence of bacteria species on the surface of the anodes was responsible for the reduction of metal as well as generating the energy output. Fig. 6 (a-f) shows the SEM images of the anodes without biofilm and with biofilm after successful operation of BMFC-I, BMFC-II and BMFC-III. The SEM images (Fig. 6 a, c, e) clearly showed that the fresh anode (before the operation starts) does not show any kind of bacterial species present on surface. After the operation, it was observed that BMFC-III showed a larger bacterial species on the used anode surface than BMFC-I or BMFC-II. Unfortunately, BMFC-II shows a very dense bacterial population but differs greatly in quantity with BMFC-I and BMFC-III. BMFC-I and BMFC-III show some filament rod structure in SEM image, BMFC-III also shows clear filament rod structure. There are several SEM analytical studies on biofilms, but rarely report the presence of filament rod structures on the anode surface (Reimers et al. 2017, Xu et al. 2016). These discrepancies may be due to different operating conditions, especially at the source of the inoculum or altered system parameters such as pH and temperature.

Organic or inorganic substrates are also important factors influencing bacterial activity. In the present work, it was observed that exoelectrogens species are showing the filamentous rod structures to transfer the electrons to anode electrode. Generally, the *Shewanella* or *Geobacter* species utilize conductive pili to transfer the electrons (Yaqoob et al. 2020b). Based on SEM images obtained from BMFC- I, BMFC- II, and BMFC- III, it can be concluded that rod-like wires are responsible for electron transfer.

Fig. 6: Biofilm morphology on the anode of (a-b) Fresh and used anode of BMFC-I (c-d) Fresh and used anode of BMFC-II (e-f) Fresh and used anode of BMFC- III.

The energy generation and electron transfer mechanisms are key factors to initiate the removal process of metal ions. In BMFC, the organic substrate is oxidized by bacterial species which generate electrons and protons during their respiration. Several exoelectrogens are well known in MFC field as energy generators (through transferring electrons) while simultaneously diminishing the metals concentration. Some examples of these bacterial are *Geobacter* sp. *Klebsiella pneumoniae*, *Acinetobacter*, *Shewanella* sp. and *Proteus* sp. etc. (Chuo et al. 2020, Yaqoob et al.

2020b). In the present study, during the BMFC operation, the bacterial species produced a biofilm (a sludge around the anode electrode), which serves as a source of microorganisms to transfer the electrons to the anode electrode from bacteria. The electrons and protons are produced by bacterial species during the oxidation process of organic substrate (Franks & Nevin 2010). This process was completed through the Krebs cycle mechanism that occurs inside the bacterial species. Chai et al. (Chai et al. 2018) reported that rambutan contains a maximum of 10.01% (sucrose), 3.18% (fructose) and 2.43% (simple sugar) and other carbohydrate content. Similarly, several studies showed that, mango and langsat also present a high sugar content (Arumugam & Manikandan 2011, Sudha et al. 2015, Techavuthiporn 2018). The organic substrate derived from this waste is sugar-based; therefore, they are believed to be efficient in promoting bacterial growth and the respiratory process. In order for these organic substrates to function as a source of carbon and energy, the conversion of complex sugars into a more moderate form (i.e., glucose) must be performed. Glucose can be further oxidized by bacterial species on the anode surface. The oxidation process can be written as:

The electrons produced during the oxidation are transferred to the anode via different mechanisms, in turn, these electrons are transferred via outer circuit. The produced protons are moved towards the cathode electrode through proton exchange membrane (Yaqoob et al. 2020a). Usually, two major type of electron transfer mechanisms are present which are following (Fig. 7):

- Direct electron transfer, which is carried out through the conductive pili (this is a filamentous wire shape that is part of some bacterial species) and redox-active proteins

(OmcZ, OmcE, OmcS, OmcB, and OmcT). This mechanism can be accomplished by the following bacteria: *Bacillus* sp., *Acinetobacter radioresistens*, *Aeromonas hydrophila*, *Klebsiella pneumoniae* etc. The *Geobacter sulfurreducens* species electron transfer mechanism is based on redox-active proteins to transfer the electrons towards the anode surface.

- Indirect electron transfer mechanism in which bacterial species utilize soluble electron's shuttles. *Geobacteraceae* and *Desulfuromonadaceae* families generally follow this mechanism.

In the present work, the SEM images (Fig 5) demonstrated the presence of rod shaped structures in which the conductive pili may be present. This indicates that bacteria that follow the direct electron transfer mechanism were present in the BMFC to transfer the electrons to the anode. Furthermore, when the electrons are successfully transferred to the cathode through the outer circuit, the reduction reaction occurs. The targeted metals (Cd^{2+} and Pb^{2+}) were then converted into insoluble form from a soluble form. The reduction reactions for the metals can be written as follows:

- Conversion of Cd^{2+} to $\text{Cd}_{(s)}$

- Conversion of Pb^{2+} to $\text{Pb}_{(s)}$

Fig. 7: Different electron transfer mechanisms during the BMFC operation.

3.9. Bacterial isolation and identification studies

The anodic biofilms of BMFC-I, BMFC-II, and BMFC-III were further analyzed through isolation and bacterial identification processes. Table 5 shows the list of the exoelectrogens that were extensively present on the surface of the BMFC-I, BMFC-II, and BMFC-III anodes. According to the observed results, almost identical bacterial species were found on the anode surfaces of BMFC-I, BMFC-II, and BMFC-III. The SEM images of the used anode also confirmed that there were a small number of bacterial species. A large number of bacteria have a filamentous structure, which suggested that conductive pili were responsible for electron transfer. The BMFC-II biofilm showed a dense bacterial population, and several different species were detected which were different from those detected on other cells. These differences may be due to environmental factors. BMFC-I and BMFC-III showed fewer bacterial species but have similar morphology. The observed bacterial

species such as *Klebsiella pneumoniae*, *Citrobacter amalonaticus* and *Enterobacter* species are well known as exoelectrogens species (Kumar et al. 2021, Munoz-Cupa et al. 2020, Wang et al. 2020, Yaqoob et al. 2020b, Zhang et al. 2020). For example, Zhang et. al., (2008) reported that *Klebsiella pneumoniae* is one of the most efficient exoelectrogens in MFC and that the maximum achieved power density obtained when this bacteria was present was of 151.11 mW/m². They stated that *Klebsiella pneumoniae* were responsible for electrons transfer through conductive pili. Uddin et al., (2020) carried out an extensive review about the metal reduction through bacterial species and they mentioned that *Klebsiella pneumoniae*, *Enterobacter*, and *Citrobacter* were metal-reducing bacterial species. According to these evidences it can be proposed that the bacterial species that were identified in the present work were responsible for electricity generation and removal of metal ions from the synthetic wastewater.

Table 5: The identified bacteria species from the anodic biofilms of BMFC-I, BMFC-II and BMFC-III.

Bacteria	Accession number1(6S rRNA Gene)	Query cover (%)	Identity (%)
BMFC-I			
<i>Klebsiella pneumoniae subsp. rhinoscleromatis</i> strain R-70	NR_037084.1	98	99.35
<i>Klebsiella pneumoniae</i> strain ATCC 13883	NR_119278.1	98	97.70

<i>Pseudocitrobacter faecalis</i> strain 25 CIT 1	NR_125690.1	98	97.70
<i>Citrobacter amalonaticus</i> strain CECT 863	NR_104823.1	98	98.21
<i>Kosakonia sacchari</i> strain SP1	NR_118333.1	98	98.20
<i>Citrobacter amalonaticus</i> strain LMG 7873	NR_118106.1	97	96.25
<i>Kluyvera ascorbata</i> ATCC 33433	NR_114589.1	96	98.05
<i>Providencia sneebia</i> DSM 19967	NR_104913.1	98	97.24
<i>Pantoea dispersa</i> strain LMG 2603	NR_116755.1	98	97.20
<i>Kosakonia radicincitans</i> DSM 16656	NR_117704.1	98	97.24
<i>Klebsiella oxytoca</i> strain NBRC 102593	NR_114152.1	98	97.07

BMFC-II			
<i>Klebsiella quasipneumoniae</i>	NR_134062.1	98	98.54
<i>subsp. quasipneumoniae</i>			
strain 01A030			
<i>Klebsiella pneumoniae</i> strain	NR_036794.1	98	97.88
DSM 30104			
<i>Enterobacter cloacae subsp.</i>	NR_118011.1	98	98.06
<i>dissolvens</i> strain ATCC			
<i>Enterobacter cloacae subsp.</i>	NR_044978.1	98	98.05
<i>dissolvens</i> strain LMG 2683			
<i>Citrobacter farmeri</i> strain	NR_024861.1	98	97.21
CDC 2991-81			
<i>Raoultella planticola</i> ATCC	NR_024996.1	96	97.84
33531			
BMFC-III			
<i>Klebsiella quasipneumoniae</i>	NR_134063.1	98	99.03
<i>subsp. similipneumoniae</i>			
strain 07A044			

<i>Klebsiella pneumoniae</i> strain	NR_112009.1	98	97.70
<i>JCM1662</i>			
<i>Citrobacter freundii</i> strain	NR_113596.1	98	97.40
<i>NBRC 12681</i>			
<i>Raoultella planticola</i> ATCC	NR_119279.1	98	97.73
33531			
<i>Yokenella regensburgei</i> strain	NR_104934.1	98	97.70
<i>CIP</i>			
<i>Kluyvera intermedia</i> strain	NR_028802.1	98	97.72
256			
<i>Serratia marcescens</i> subsp.	NR_041980.1	98	97.56
<i>marcescens</i> ATCC 13880			
<i>Gibbsiella dentisursi</i> strain	NR_118387.1	98	97.72
<i>LEN33</i>			

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491 **4. Conclusion, future challenges, and perspectives**

492 BMFC opened a new door of research in the field of microbial fuel cells that may positively
493 lead to an environmentally friendly, sustainable, easily controllable, and cost-effective approach
494 to energy generation and wastewater treatment. Increasing evidence has been developed in recent

years to demonstrate the BMFC significance for improving the natural bioremediation practices. BMFC still needs attention to perform at a larger scale due to several challenges. According to the literature review, it was observed that most of the efforts have been focused on development of electrodes in BMFC to enhance the energy output or improving bioremediation process. In BMFC the performance of the electrodes is an important aspect but there are other important aspects that must be considered for a successful BMFC performance, i.e., organic substrate or waste used in the BMFC as fuel for bacterial activities. In the present work, the focus is on the organic substrate, which is well known as a fuel for bacteria species to generate the energy in the BMFC, which leads to a successful green technology. The present study targeted three local Malaysian fruit wastes which are available in large amounts in the country as well as several other Southeast Asia nations. The utilization of waste material as organic substrate was an ideal approach to generate bioenergy and toxic metals removal. Currently, Malaysia faces the problem of the presence of toxic metals in water resources, especially the presence of Cd^{2+} and Pb^{2+} .

The operation of the system in this work was performed in three separate but identical preparations for 45 days until the bacterial species entered the decaying phase due to insufficient energy supply. The maximum CD produced in BMFC-I, BMFC-II and BMFC-III was of 36.84 mA/m^2 , 39.47 mA/m^2 and 87.71 mA/m^2 , respectively. These values were achieved by varying the different external resistances from 150-7 $\text{k}\Omega$. Out of the three fruit wastes, the mango waste (peel and seed waste) was the preferred as fuel for bacteria due to the presence of a high sugar content. The sugar content is the energy source for bacterial growth and respiration. Similarly, the removal efficiency of Cd^{2+} and Pb^{2+} were also successfully achieved. The BMFC-I presented 60 % (Cd^{2+}) and 68 % (Pb^{2+}) removals, BMFC- II presented 69 % (Cd^{2+}) and 71 % (Pb^{2+}) removals and BMFC- III presented 78 % (Cd^{2+}) and 80 % (Pb^{2+}) removals. According to the experimental outcomes

obtained on the energy generation and metals removal, it was important to optimize the pH and temperature. It was found that the neutral pH and room temperature are the most favorable conditions to create an environment for biofilm growth and promoting metabolic activities.

According to present observations, it manages to produce a reasonable energy output in a relatively short time compared to the previous works, but its metal removal rate is relatively low. The reason for the low removal efficiency is the instability of the fruit waste for long periods of time. Decreased waste causes a decrease in energy supply over time which affects bacterial activity. Therefore, future research should be done on different wastes that can provide long-term energy supply to bacteria for more effective removal efficiency. Different waste materials such as sugar cane waste, durian (*durio*) waste, potatoes waste etc. are expected to address the long-term energy supply issue. Research on the production of efficient electrodes and organic substrates for BMFC could result in better metal removal efficiencies. Metal removal is almost never studied in BMFC compared to sedimentary microbial fuel cells or MFC. Thus, promoting the use of BMFC as a safe and green approach requires a comprehensive and continuous effort.

Author contribution

Mohamad Nasir Mohamad Ibrahim, Khalid Umar: Conceptualization. Asim Ali Yaqoob, Khalid Umar: Methodology, writing-original draft preparation, visualization. Amira Suriaty Yaakop electromicrobiology investigation. Claudia Guerrero–Barajas: English editing of the manuscript. Mohamad Nasir Mohamad Ibrahim: Supervision, funding acquisition. This article has been read and approved by all listed authors.

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Data Availability Statement

The authors confirm that all data underlying the findings are fully available without restriction.

Data can be obtained after submitting a request to the corresponding/first author.

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