

Microbial Fuel Cell's Performance of Original and Deoxygenated Palm Oil Mill Effluent in 3 Different Stages of Fermentation Process

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ABSTRACT

Bio-electricity generation by Microbial Fuel Cell (MFC) has gained considerable attention due to its integration with wastewater treatment such as Palm Oil Mill Effluent (POME). Investigation into pH effect and determination of optimal pH value ranges growth for acidogenic, acetogenic

and methanogenic by natural mixed culture electroactive bacteria (exoelectrogens) growth in original non-Deoxygenated Mixed POME (nDMP) and Deoxygenated Mixed POME (DMP) in MFC was carried out. Current generation, power generation and maximum power were also monitored. Experimental results show that exoelectrogens in nDMP with pH 6.8 yielded the highest current generation of 61.51 mA^m-² and maximum power of 17.63 mWm⁻². Overall, nDMP substrates with 3 pH ranges (5.5, 6.8 and 8.0) showed equal potential to generate power that is higher than DMP substrates. Comparison carried out for inter DMP substrates demonstrated that DMP with pH 6.8 and DMP with pH 8.0 showed

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equal potential to generate power, but not for DMP with pH 5.5. Subsequently, nDMP with pH 6.8 and nDMP with pH 8.0 showed equal potential for higher maximum power compared to nDMP with pH 5.5 and DMP substrates. This finding indicates that mixed microbial communities in DMP substrate are dominant with obligate anaerobic exoelectrogens bacteria which have less capability to generate electricity compared to nDMP substrate that was dominated by the aerotolerant and/or facultative anaerobic exoelectrogens bacteria.

Keywords: Microbial Fuel Cell (MFC), Palm Oil Mill Effluent (POME), pH value

INTRODUCTION

In Malaysia, POME is a highly polluted wastewater produced by palm oil processing mills, which was usually treated in open pond system consisting of cooling ponds, acidification ponds, anaerobic ponds and facultative ponds until its biological oxygen demand (BOD) meets the limit set by Malaysian Department of Environment (DOE) which is 5000 mg/l (Environmental quality act 1974, 1978) before discharging it into the water course (Andrew & Manaf, 2013).

POME anaerobic fermentation is a complex biochemical process in anaerobic condition, where organic matters are degraded to methane and carbon dioxide in discrete steps involving the concerted action of a myriad numbers of bacteria in several different metabolite groups of microorganism. The main pathways of anaerobic digestion are involved in 4 stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Andrew & Manaf, 2013).

During hydrolysis, large polymers of carbohydrate, lipids (fat) and protein macromolecules were broken down to amino acids, long-chain fatty acids, and sugars. These hydrolysates were then further fermented during acidogenesis to produce three, four and five-carbon volatile fatty acids, where optimum pH range growth for acidogenic bacteria was between pH 5.2 to pH 6.5. These hydrolysates (products of hydrolysis process) were consumed by acetogenic bacteria and generated acetate acids, carbon dioxide and hydrogen during acetogenesis, where optimum pH growth of these bacteria was between pH 6.0 to pH 7.0. Finally, during methanogenesis, where methanogenic bacteria's pH growth environment was within the range of pH 7.5 to pH 8.5 consumed acetate, hydrogen and some of the carbon dioxide to produce methane (Rappport et al., 2008; Solera et al., 2002).

Apart from the above categorisations, bacteria may be divided into three groups according to their responses to free molecular oxygen. These groups are: (1) strictly aerobes; (2) facultative anaerobes; and (3) anaerobes which are inactive in the present of free molecular oxygen which may be divided into two subgroups; oxygen-tolerant species and oxygen-intolerant species. Anaerobic bacteria present in POME anaerobic pond could be grouped on the basis of their need for oxygen to grow. Obligate anaerobic bacteria (oxygen-intolerant species) are the bacteria that use anaerobic metabolism to grow but harmed and killed in the presence of oxygen. Meanwhile, aerotolerant anaerobic bacteria (oxygen-tolerant species) are also the bacteria that also use anaerobic metabolism to grow but could tolerate the presence of oxygen. The two subgroups mentioned above need anaerobic metabolism to grow, but not for facultative

anaerobic bacteria. Facultative anaerobic bacteria prefer to grow using aerobic metabolism processes but could switch to anaerobic metabolism in the absence of oxygen (Gerardi, 2003).

Recently, POME treatment process involving bacteria has gained some interest amongst scientists and environmentalists, and usage of bacteria together in Microbial Fuel Cell (MFC) is one of the alternatives for POME treatment. Meanwhile, the application of MFC technologies is an alternative approach to wastewater treatment that uses by-product generated electricity (Logan, 2008). In 2004, the relationship between electricity using MFCs and wastewater treatment was clearly forged when it was demonstrated that domestic wastewater could be treated to practical levels while simultaneously generating electricity (Liu & Logan, 2004). Bioelectricity generation is a new approach for generating electricity from biomass using bacteria. Therefore, bacteria were further categorised by their ability to exogenously transfer electrons called exoelectrogens that can produce power in an MFC (Logan, 2008). Other names for exoelectrogens are electrogens, anode-respiring bacteria (Torres et al., 2010) and electrochemically active bacteria/microorganism (Samir et al., 2010). Exogenously transfer electrons by bacteria are also known as e Extracellular Electron Transfer (EET) (Torres et al., 2010).

In this work, mixed POME substrates (1:1, Raw POME: Anaerobic Pond POME) were subjected into 2 factors that may influence the growth of different species of bacteria. The first factor was the pH of substrate, which was controlled into three groups of pH (pH 5.5, 6.8 and 8.0) that was the optimal pH for acidogenic, acetogenic and methanogenic bacteria (Solera, Romero, & Sales, 2002). The second factor was the dissolved oxygen (DO) in the substrate. Two types of substrates were used; one was pretreated by sparging with pure nitrogen gas (N₂) (Logan, 2008) to decrease DO, while the other substrate was not pretreated. These factors were investigated in terms of the pH effect to determine the optimal pH value ranges growth for acidogenic, acetogenic and methanogenic by the exoelectrogens growth in nDMP and in MFC. In addition, the percentage of COD effective reduction was used as the indicator to determine POME treatment by the MFC. Common approaches used in most studies are entirely relying on its pH values and tend to ignore the pathways of anaerobic digestion, in which anaerobic digestion process is as important as pH values. By solving this problem, the understanding of bacteria's activity respectively to their environment, especially in acidogenesis, acetogenesis and methanogenesis stages, is enhanced.

METHOD

In this work, the double-chambered MFC was designed and fabricated locally using acrylic fibre material with 150 mL working volume for each component. The anode (anaerobic) and cathode (aerobic) chambers were separated by a salt bridge compartment filled with molten 10% agarose of 4% potassium chloride salt (Nair et al., 2013) heated in a water bath, which was allowed to be cooled and solidified. The salt bridge assists in the proton transfer mechanism during MFC's operation. The electrode material used for anode and cathode was carbon brush (Bosch Skil Dremel PG88, German) in the sizes of 2.2 cm x 1.6 cm x 0.6 cm (used in both chambers) and 1.3 cm x 0.7 cm x 0.6 cm (used only in anode chamber) with projected area of 62.22 cm² for anode and 11.6 cm² cathode. The anode chamber is sealed to maintain its

anaerobic condition. The electrodes close circuit was connected externally through copper wires to provide the permanent connection to external resistance of 1 k Ω resistor. A schematic diagram of MFC is shown in Figure 1.

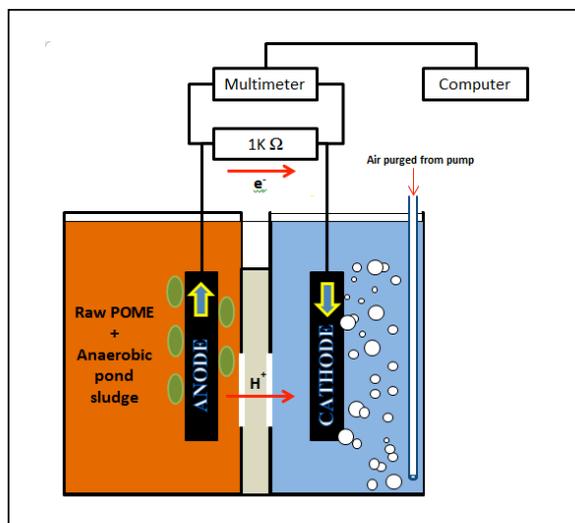


Figure 1. A schematic diagram of aqueous cathode double-chamber MFC

The substrate used is mixed POME. The substrates were prepared in 2L beakers at a mixing ratio of 1 L raw POME to 1 L of anaerobic pond POME. Gas flushing or sparging pure N₂ gas (Logan, 2008) through the mixed POME (Deoxygenation pretreatment) was a process to decrease DO (Japar et al., 2013; Biffinger et al., 2008; Logan et al., 2006) in the substrate. The pretreatment was applied to the first mixed POME sample mixture, DMP, while the second was not, nDMP. The two beakers were sealed with parafilm. After 24 hours, the substrates were divided into six units of 250 mL borosilicate glass reagent bottles (Bomex), where three of the bottles were filled with DMP and the rest with nDMP.

The pH of substrates were controlled by using sodium hydroxide (NaOH) that maintained pH 5.5 ± 0.2 , which represented DMP pH range between 5.2 to 6.5 (DMP 5.5) and nDMP pH range between 5.2 to 6.5 (nDMP 5.5). The same procedure was done to both of the substrates, DMP and nDMP, to maintained pH 6.8 ± 0.2 and pH 8.0 ± 0.2 (DMP 6.8, nDMP 6.8, DMP 8.0 and nDMP 8.0). All the six controlled substrates were left for 24 hours before filling the anode chambers 125 mL of mixture. The balance of 125 mL POME batches were kept sealed in the borosilicate glass reagent bottles (Bomex) as controlled and calibrate samples.

The electrode output voltage was recorded every 15 minutes with a digital multimeter with a data logger (DM620, 50,000 Count Logger DMM and UT803, UNI-T). From these

data, power generation was calculated using power density normalised by the surface area of anode (P_A , Wm^{-2}) (Logan et al., 2006) using the equation [1] below:

$$P_{An} = \frac{V^2}{A_{An}R} \quad [1]$$

Where A = total area of anode electrode (m^2), An = Anode, P = power (W), V = the potential (V) and R = external resistance (Ω). The polarisation curves represent the voltage as the function of the current (density) was used for the analysis and characterisation of MFC (Logan et al., 2006), whereas the power curve described the power (or power density) as the function of the current (or current density) calculated from the polarisation curve. The power increased with the current from open circuit conditions to maximum power point (MPP). From the previous works, researchers typically use the peak (top) of the power curve, which is the MPP, to report the "Maximum Power" and this maximum power occurred at the point where the internal resistance was equal to the external resistance (Logan, 2008). To obtain the maximum power data from the power curves and plot the polarisation curve, variable resistance using the resistors MODEL: ITC 8 Insulation Tester Checker (KK Instruments, Taiwan) was applied in the MFC circuit by connecting it to different resistors (0.5, 1, 2, 20, 200, 1k, 10k, 20k, 100k, 1M, 2M and 10M) Ω and voltage reading was recorded (typically 10 to 20 min per resistor). From these data, power density and current density were calculated. The current density normalised by surface area (I_{An} , Am^{-2}) was calculated using equation [2] below:

$$I_{An} = \frac{V}{A_{An}R} \quad [2]$$

Where A = total area of anode electrode (m^2), An = Anode, V = the potential (V) and R = external resistance (Ω) and I = current (A). From this calculation, polarisation curves and current density curves were plotted (Logan et al., 2006).

Environmental Scanning Electron Microscopy (Quanta 450 FEG ESEM, USA) was used to observe the biofilm formed on the anodic carbon brush's (ACB) surface. A cut ACB was soaked for 4 hours in 4% glutaraldehyde phosphate buffer solution for fixation process. Then, the ACB was rinsed in distilled water before the sample was observed under ESEM at 20 kV.

RESULTS AND DISCUSSION

Voltage reading was recorded, where power and current densities were calculated by the MFC devices for DMP, and nDMP substrates with 3 different ranges of pH values showed that the power and current generation by nDMP substrates was higher compared to the DMP substrates. The voltage of nDMP 6.8 was relatively high as compared to nDMP 5.5 and nDMP 8.0, as shown in Figure 2. The same was applied to DMP, whereby DMP 6.8 recorded the highest voltage compared to the other two DMPs. The lowest power generation calculated for DMP substrate was DMP 5.5, which was almost to none after 10.5 hours of MFC's operation, and the lowest for nDMP substrates was nDMP 5.5, respectively. The highest point for the calculated power for nDMP was nDMP 6.8 at 14.76 mWm^{-2} after 102.5 hour of MFC operation, while for DMP was DMP 8.0 at 5.04 mWm^{-2} after 14.5 hour of MFC operation (Figure 2). The result shows

that pH value does affect MFC's power generation performance. The anodic pH value ranged between 6.8 ± 0.2 for both DMP and nDMP substrates produced the highest power generation as compared to the other two, pH value ranges between 5.5 ± 0.2 and 8.0 ± 0.2 , respectively, where nDMP 6.8 substrates recorded the highest power generation. This is in agreement with Patil et al. (2011) whose previous study indicated that pH value played a crucial role for the development and current production of anodic microbial electroactive biofilm, whereby only a narrow pH window from pH 6.0 to 9.0 was demonstrated to be suitable for the growth and operation of biofilm, and that a high average of current density was achieved at pH 7.0.

nDMPs power generations are relatively higher than DMPs, as shown in Figure 2. This proves that MFC's performance is not just affected by pH value but also by the different concentrations of DO. It is possible that the capabilities of the dominant mix community's anoxic exoelectrogens in DMP to generate electricity were lower compared to dominant mix community's aerotolerant and/or facultative bacteria in nDMP. Facultative bacteria have the ability to metabolise and produce electricity in both the presence and absence of oxygen, which gives the bacteria higher survival rate than obligate bacteria. In a previous study, the experiment of *Shewanella oneidensis*'s electrical performance in MFC by exposing the bacteria in different oxygen concentration was conducted, and the researchers concluded that *S. oneidensis*'s electrical performance in aerobically condition was higher than anaerobically condition, which is similar to the bacterial behaviour against concentration of hydrogen ion (Biffinger et al., 2008).

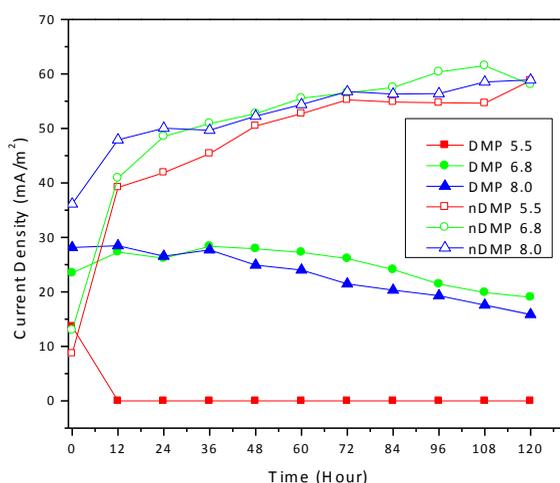


Figure 2. Power Generations with DMP and nDMP exoelectrogens bacteria by pH value

Anaerobic mix culture bacteria presence in the substrate used in this study could be divided into Obligate Anaerobic Bacteria (oxygen-intolerant species), Aerotolerant Anaerobic Bacteria (oxygen-tolerant species) and Facultative Anaerobic Bacteria that responded to the DO (Gerardi, 2003). Deoxygenation pretreatment with pure N_2 gas to reduce DO content makes the environment of DMP from hypoxic to anoxic. In this condition, obligate bacteria grow and

become dominant in number compared to aerotolerant and/or facultative bacteria. Therefore, DO concentrations could determine the active species of bacteria and their populations in the mix cultured POME as shown in Figure 5, which is in agreement with Biffinger et al. (2008).

At $1k\ \Omega$ external resistance, DMP 6.8 recorded the maximum power that MFC system could generate at power density peak at $2.92\ \text{mWm}^{-2}$ (current density = $21.65\ \text{mA}\text{m}^{-2}$) as compared to DMP 5.5 peak at $0.15\ \text{mWm}^{-2}$ (current density = $4.95\ \text{mA}\text{m}^{-2}$) and DMP 8.0 peak at $2.09\ \text{mWm}^{-2}$ (current density = $18.33\ \text{mA}\text{m}^{-2}$) as illustrated in Figure 3 below.

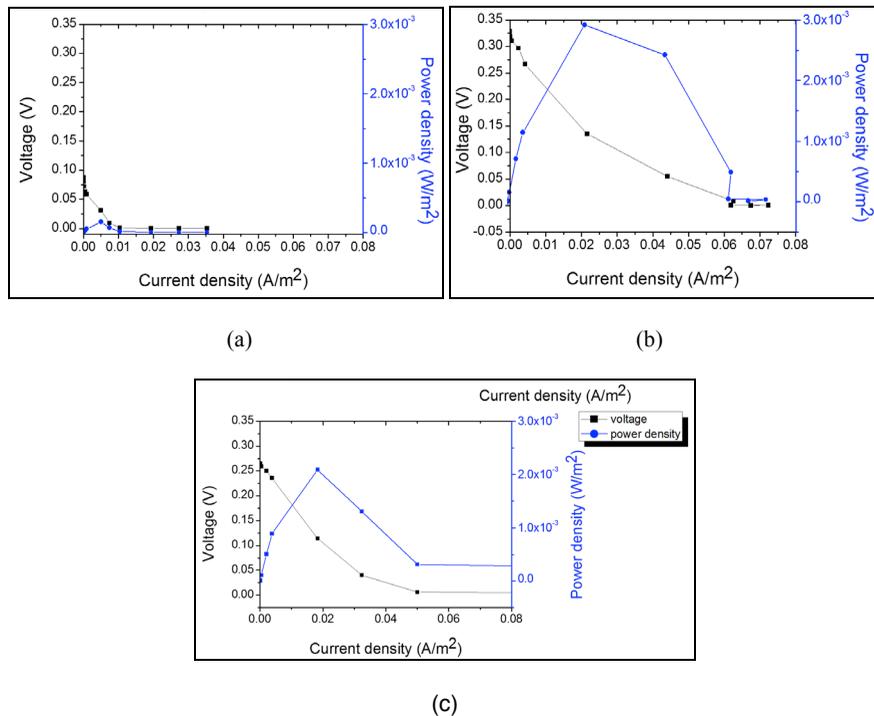


Figure 3. Polarisation and Power Curves with: (a) DMP 5.5; (b) DMP 6.8; and (c) DMP 8.0

To demonstrate the potential for greater power densities, further test was conducted with nDMP substrates. The maximum power of nDMP 5.5 was at $1K\ \Omega$ external resistance, but nDMP 6.8 and nDMP 8.0 were optimum at the external resistance of $200\ \Omega$ for the other two MFC systems. At their respective external resistance, the nDMP 6.8 recorded the highest maximum power whereby the power density peak at $17.63\ \text{mWm}^{-2}$ (current density = $150.28\ \text{mA}\text{m}^{-2}$) as compared to nDMP 5.5 which peaked at $13.84\ \text{mWm}^{-2}$ (current density = $59.56\ \text{mA}\text{m}^{-2}$) and nDMP 8.0 peaks at $15.13\ \text{mWm}^{-2}$ (current density = $139.24\ \text{mA}\text{m}^{-2}$), as shown Figure 4.

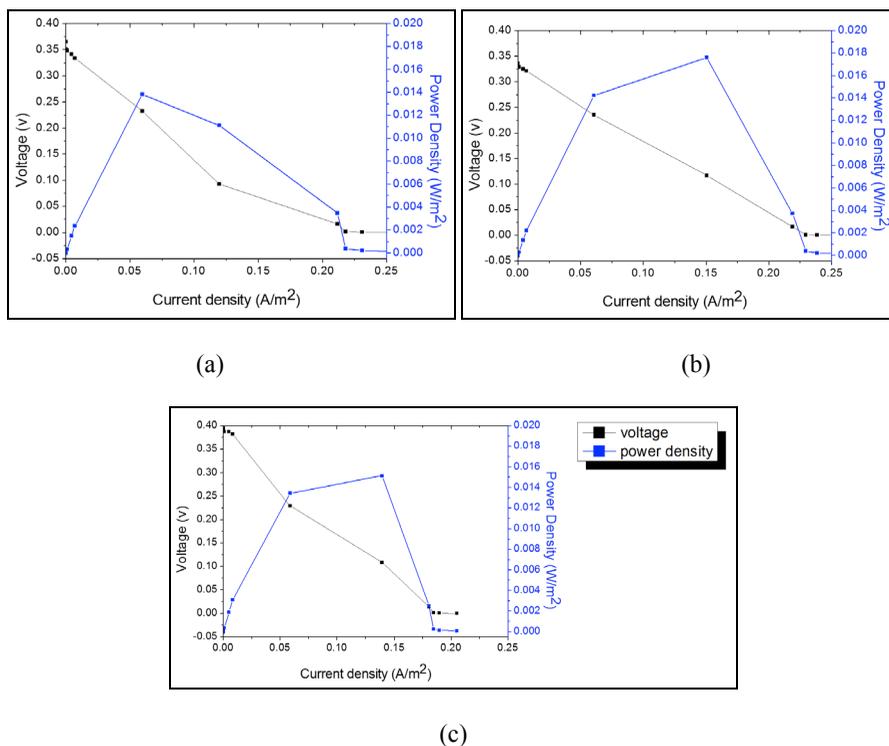


Figure 4. Polarisation and Power curves with: (a) nDMP 5.5; (b) nDMP 6.8; and (c) nDMP 8.0

Overall, the maximum power that the MFC system of nDMP substrates produced was higher than the MFC system of DMP substrates. For inter pH ranges, the maximum power of nDMP 5.5 was 92 times higher compared to DMP 5.5. Meanwhile for nDMP 6.8, the maximum power was 6 times higher compared to DMP 6.8 and nDMP 8.0, and it was 7 times higher compared to DMP 8.0. However, for intra pH ranges, nDMP 6.8 substrates recorded the highest maximum power. The maximum power of nDMP 6.8 was 1.17 times compared nDMP 8.0 and 117.5 times compared to DMP 5.5.

The anodic pH values range between 6.8 ± 0.2 for both DMP and nDMP substrates which produced the highest maximum power as compared to the other two pH value ranges between 5.5 ± 0.2 and 8.0 ± 0.2 , respectively where nDMP 6.8 substrates recorded the highest power generation. This is in accordance with Patil et al. (2011).

Acidogenic (exoelectrogens) bacteria did not grow in Alkaliphile condition but grew in Neutrophile condition. However, the presence of acidogenic (exoelectrogens) bacteria in Neutrophile condition did not increase the maximum power demonstrated in the result above (Figure 3 and Figure 4). At this point, the acidogenic (exoelectrogens) bacteria are at the optimum, thus anodic pH ranges recorded the lowest. Although acetogenic (exoelectrogens) bacteria active in DMP 6.8 and nDMP 6.8 substrates, but this does not mean that it is exclusive for these bacteria only. Some alkaliphilic bacteria, which are methanogenic (exoelectrogens) bacteria, grow in the pH value ranges between 6.8 ± 0.2 , a similar behaviour observed in Ziemiński and Frąc (2011). The fact that the conversion of fermentation product by acetogenic

(exoelectrogens) bacteria was thermodynamically possible only if the hydrogen concentration was kept sufficiently low by converting it into methane by methanogenic (exoelectrogens) bacteria. Therefore, the acetogenic and methanogenic (exoelectrogens) bacteria must cohabit in a close relationship between both classes of bacteria. This conversion of the fermentation is in agreement with Solera et al. (2002).

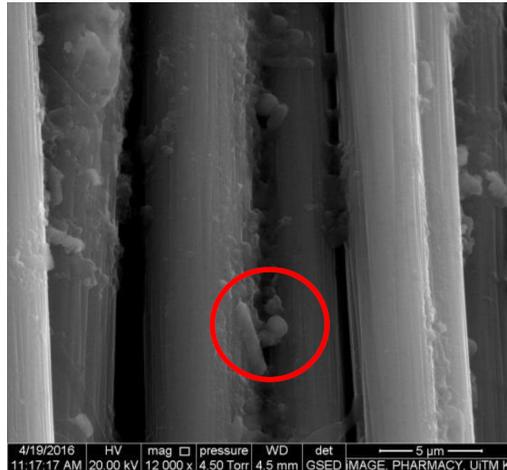


Figure 5. ESEM images of microorganisms' attachment on ACB's surface at day 5

Environmental Scanning Electron Microscopy (ESEM)

ESEM was used to examine the ACB's surface to confirm the presence of biofilm formation. On day 5 of MFC operation, the ACB was observed under ESEM and the result is as shown in Figure 5. In Figure 5, all sorts of bacteria can be observed on the ACB's surface. The figure illustrates that the rod-shaped and round-shaped bacteria are attached (red circle) on the surface, which indicates the biofilm formed on the ACB's surface came from a mixed culture bacteria POME sample.

CONCLUSION

The pH of 6.8 ± 0.2 representing the pH range between pH 6.0 and pH 7.0 was found to be the optimal condition for acetogenic bacteria growth in both DMP and nDMP. However, nDMP produced the highest power generations and maximum power as compared to the DMP, 17.63 mWm^{-2} and 2.92 mWm^{-2} , respectively. Additionally, the nDMP substrates for all pH ranges show equal potential to generate power, and the DMP substrates for pH 6.8 and pH 8.0 also have the same potential. Nevertheless, bacteria in nDMP substrates produced relatively higher power generation and maximum power compared to bacteria in the DMP substrates.

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