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Stacked configuration effect on microbial fuel cell performances via acid red 27 dye biodecolourisation

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ABSTRACT

In this research, three individual Microbial Fuel Cell (MFC) units were connected in series and parallel for stacked MFC performance assessment by using the bacterial consortium (Citrobacter freundii A1 and Enterococcus casseliflavus C1) for bioelectricity generation through Acid Red 27 (AR-27) decolourisation in modified P5 medium (2.5 g/L glucose and 5.0 g/L nutrient broth). For the decolourisation efficiency, both MFCs in series and parallel connections achieved more than 90% decolourisation at a temperature range of 29 ± 2°C by using the bacteria consortium. The FTIR analysis illustrated that the Azo bond was completely cleaved after the decolourisation process. Through the characterization of microbial fuel cell, in series mode, the maximum open circuit voltage (OCV) recorded was 365 mV. While for the maximum close circuit voltage (CCV) was 244 mV, when an external load of 100 kΩ was introduced. The current density achieved in series mode was 0.871 µA/cm2 which is almost double from that of in parallel mode, which was 0.441 µA/cm2. The bioelectricity power generated in series and parallel was 212.629 µW/cm2, and, 54.384 µW/cm2, respectively. This finding suggests that bioelectricty generation through decolourisation of azo dye was plausible with the bacteria consortium combination. In term of bioelectricty generation, MFCs aligned in series mode was able to be achieved higher voltage, current density and power density compared to single unit MFC and MFCs in parallel mode.

Keywords:
Microbial fuel cell, azo dye decolourisation, bioelectricity, series and parallel, power density, current density

1. Introduction

According to the Environmental Quality Act 1974 [1], water pollution is defined as any direct or indirect changes to the physical properties, thermal, biological, or radioactive that any part of the environmental release to the water. These may cause dangerous situation and detriment of health,

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safety and welfare of the public, or the lives of other organisms, such as birds, wildlife, fish and aquatic life and water plants.

One of the major factors that contribute to water pollution is the wastewaters containing dye discharged by the textile industries. These dyes were lost in the effluent during the dyeing process and these dyes are highly stable and resistant to microbial degradation. Therefore, it makes it not easily degradable under natural environment. Moreover, the conventional wastewater treatment systems do not typically remove the dyes in the wastewater [2] and the treatment process generates secondary pollution problems and producing concentrated sludge as by-products in which difficult to be disposed [3]. Azo dyes, which are aromatic compounds with one or more azo bond (–N=N-) groups, are the most commonly used synthetic dyes in commercial applications such as textile industry [4]. Recent studies have shown that azo dyes contribute to the mutagenic activity of ground and surface water pollution by textile effluents [5].

Current treatment such as physio-chemical method has been applied to treat dye containing wastewater. Although high rates of dye removal can be efficiently achieved, physico-chemical methods have some disadvantages including high cost and sensitivity [6]. Moreover, complete dye degradation could not be achieved by normal treatment that only involve aerobic biological process [7]. Alternatively, biological processes may provide a less expensive and efficient alternative for simultaneous colour and organic matter removal from dyes containing wastewater. Recently, a new technology called Microbial Fuel Cell (MFC) has been introduced as alternative solutions for both wastewater treatment and electricity generations [8, 9]. This Microbial Fuel Cell (MFC) technology represents a simultaneous alternative form of energy where in wastewater and industrial effluents [9, 10] are used for electricity generation compared to conventional wastewater treatment that requires high energy consumption to operate. Microbial Fuel Cell (MFC) applied the principle of converting chemical energy to electrical energy by the microorganism's catalytic activities [11].The application of MFCs will enhance the decolouration of azo dyes and simultaneously harvests the electricity from biodegradable organic carbon source.

Although Microbial Fuel Cell (MFC) has the potential to be assimilates with wastewater treatment, the amount of electricity generations by this technology is still not up to the par. Thus, the objectives of this study is to set up and assess the ability of the dual chamber MFCs for azo dye decolouration and bioelectricity generation by using azo dyes degrading microorganisms consortium consists of Citrobacter freundii A1 and Enterococcus casseliflavus C1. Initially, the study will focus on preparation of bacteria mixed culture for the azo dye decolourisation study. Then, followed by the preparation of MFC set up and performances study for the bioelectricity generation were tested as single unit and stack MFC units (series and parallel). The reduction of azo dye decolourisation was analysed by using electrochemistry analysis (Cyclic Voltametric) and the characterisation of biofilm formation on MFC anode was assess by using Scanning Electron Microscope (SEM).

2. Materials and Method

2.1 Bacteria Culture and Medium

Two local bacterial isolates belonging to the family Enterobacteriaceae, namely Citrobacter freundii strain A1 (AKTT01000000) and Enterococcus casseliflavus strain C1 (AKKS01000000) were used in this study. These two bacteria were obtained from the Microbiology Laboratory of the Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia. Initially, these microorganisms were grown on nutrient agar (Merck, Germany) at 37 °C. Then, P5 medium containing K_2HPO_4 (35.3 g /L), KH_2PO_4 (20.9 g/ L), NH_4Cl (2 g/ L), glucose (10 g/ L), nutrient broth (20

g /L) and trace elements was prepared according to the procedures described by previous study [12]. To prepare the starter culture, a single bacterial colony of C. freundii strain A1 and E. casseliflavus strain C1 was inoculated into a 250-mL conical flask containing 50 mL of P5 medium and incubated overnight at 37 °C with shaking at 200 rpm for 16 hours, respectively. Optical density (OD) of each bacterial culture was determined at 600 nm (GENESYS 10s UV–Vis Spectrophotometer). The inocula were ready to be used for both decolourisation efficiency and bioelectricity generation when the absorbance reading reached 1.0 ± 0.2.

2.2 Decolourisation of Azo Dye

Acid Red 27 (AR-27) dye, (Sigma Aldrich) (Figure 2) was used for the azo dye decolourisation study to determine the effects of pure and mixed culture of *C. freundii* A1 and *E. casseliflavus* C1 toward the azo dye decolourisation efficiency.

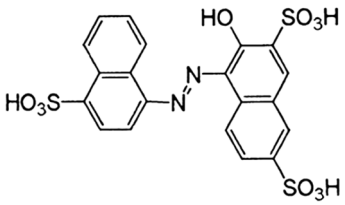


Fig. 1. Amaranth (AR 27) molecular structures

Initially, the azo dye decolourisation was conducted in flask (250 mL) that consists of 50 ml of modified P5 medium with AR-27 dye (0.1 g/L) and was mixed with 1 ml of pure and mixed culture of *C. freundii* A1 and *E. casseliflavus* C1 in a facultative anaerobic condition. The samples were then incubated in an incubator shaker at 37ºC for 2 hour. Sample collection was performed at a 10 minutes interval. Each sample was centrifuged and the supernatant were analysed using a UV-Vis spectrophotometer (GENESYS 10s UV-Vis at 521 nm to determine the level of decolourisation. The decolourisation efficiency was determined according to following relationship

$$\text{Decolourisation efficiency (\%)} = \frac{(Ai-Af)}{Ai}\ x\ 100\% \quad (1)$$

where Ai refers to the initial absorbance of dye prior to operation, Af refer to the final absorbance at any time. Then, the qualitative analysis of Fourier Transform Infrared (FTIR) spectroscopy (Thermo Scientific iD7 ATR) was performed to determine the azo bond spectrum before and after decolourisation using the selected bacteria culture.

2.3 Microbial Cell Configuration

The dual chamber MFC were set up according to previous study [13] with modifications. The anaerobic (anode) – aerobic (cathode) chamber were connected to the multimeter by using a copper wire. Then, the mixture of amaranth dye (0.1 g/L), inocula (10% v:v) and modified P5 media was placed into the anode chamber while phosphate buffer was placed into the cathode chamber with a total working volume of 100 m. The compartment used for the chambers were 5 cm × 5 cm× 9 cm with a total volume of 200 ml. The anode and cathode are separated by salt bridge (10% agarose, 1 M NaCl) for proton exchange. Copper rod (7 cm) was used to hold the electrode whereby

a carbon felt with the size of 1 cm × 1 cm × 0.2 cm with the surface area of 2.8 cm^2 were attached on the copper rod. For the comparison study of type of MFC connections, the MFCs were connected in series and parallel connection separately as shown in Figure 1 for the electricity generation analysis. For this study, both series and parallel connections required three sets of MFC while single unit MFC was used as control.

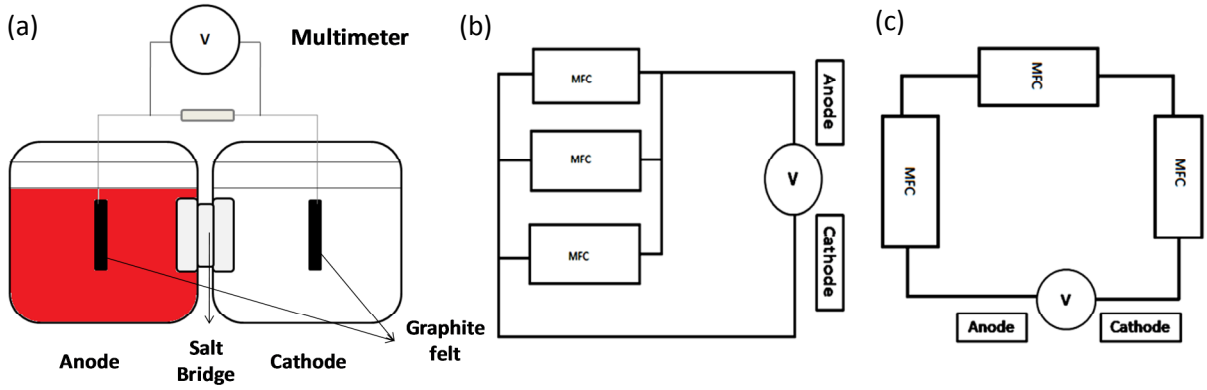


Fig. 2. Schematic diagram of Microbial Fuel Cell (MFC) set up and operation in single unit (a) and connections configuration: Parallel (b) and Series (c).

2.4 Microbial Fuel Cell Operation

The bioelectricity generation was investigated from a synthetic wastewater model of amaranth dye (0.1 g/L) in MFC. Mixed culture of *C. freundii* A1 and *E. casseliflavus* C1 were used for the determination of decolourisation rate and electricity generation measurements. For this analysis, the MFCs were connected in series and parallel cofiguration separately. Then, the MFCs were run at temperature range of 29 ± 2ºC to measure the Open Circuit Voltage (OCV) and Closed Circuit Voltage (CCV). The data was collected using a digital multimeter every 24 hours interval for 7 days. The Close Circuit Voltage (CCV) was measured across four different resistor values (10 Ω, 100 Ω, 5 kΩ and 100 kΩ) as the external loads. The current (I) and power (P) density were normalized to the anode surface area (2.8 cm^2). Projected surface area is determined accordingly based on the following equations

$$I = V/R \quad (2)$$

$$P = V*I \quad (3)$$

2.5 Cyclic Voltammetry Analysis of Azo Dye

Cyclic voltammetric experiments were carried out employing a three-electrode configuration consisting of a glassy carbon as a working electrode and calomel standard electrode as the reference. All potentials given are related to this reference electrode. While, glassy carbon was used as counter electrode. The electrochemical measurements were performed using a potentiostat (Autolab Potentiostat / Galvanostat) with 50 mV/s scan rate with the calibration of cyclic voltametry potentiostatic to determine the redox properties of the azo dye decolourisation. The result was analysed using NOVA 1.1 software.

2.6 Biofilm Morphological Analysis

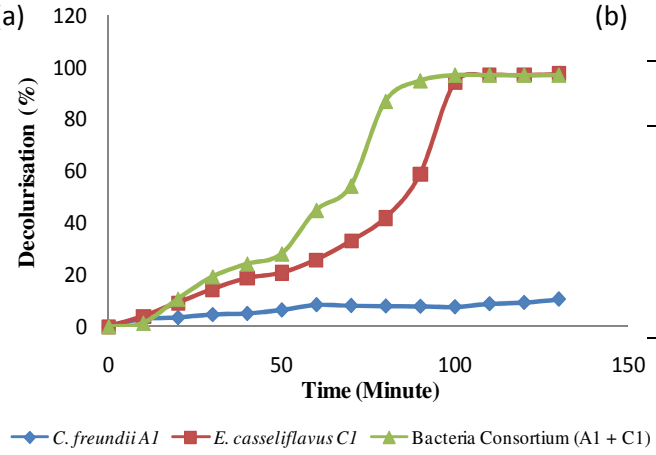
The biofilm analysis for MFC anode electrode was performed by Scanning Electron Microscope (SEM) (Hitachi Tabletop Microscope TM3000) was to examine the surface morphology of the electrode after decolourisation of amaranth dye and MFC operation. The untreated graphite felt was used as control to compare the electrode surface before being treated in the MFC anode chamber.

3. Results and Discussions

3.1 Effects of Pure and Mixed Culture Bacteria in Dyes Decolourisation

The effects of pure and mixed culture of *C. freundii* A1 and *E. casseliflavus* C1 were determined for the highest degree of decolourisation rates and efficiency and the results was illustrated in Figure 3(a). in this study, the absorbance readings indicated that the azo dye concentration and colour intensity were reduced with time. Bacterial consortium consisting of *C. freundii* A1 and *E. casseliflavus* C1 has higher decolourisation efficiency compared to pure bacterial culture of *C. freundii* A1 and *E. casseliflavus* C1. Furthermore, Figure 3(b) indicates that the bacterial consortium of *E. casseliflavus* C1 and *C. freundii* A1 has the ability to decolourize the amaranth dyes within 120 minutes with 97.5% decolourisation efficiency at 29 ± 2ºC.

(a)



(b)

Type of Bacteria Culture	Percentages of Decolourisation (%)
C.freundii A1	10.7
E. casseliflavus C1	97.0
Mix Culture (*C. freundii* A1 and *E. casseliflavus* C1)	97.5

Fig. 3. (a) Decolourisation efficiency comparisons between pure culture and mixed culture of *C. freundii* A1 and *E. casseliflavus* C1, (b) Maximum decolourisation percentages after 2 hour facultative anaerobic treatment

When grown as single pure culture in the same condition, the decolourisation efficiency of *E. casseliflavus* C1 and *C.freundii* A1 was 97% and 10.7%respectively. This results shows that both bacteria strains has azo dye decolourisation properties. Previously, NAR-2 bacteria consortium (*E. casselifalvus* C1, *E. Clocae* L17, and *C. freundii* A1) and NAR-1 (*E. casselifalvus* C1 and *E. Clocae* L17) was founded to be able decolourise azo dye such as Acid Red 27 (AR27) and Acid Orange 7 (AO7) with more than 90% decolourisation efficiency [12,13]. Although both bacteria strain was used in NAR-1 and NAR-2 bacteria consortium, the performances of *E. casseliflavus* C1 and *C.freundii* A1 as consortia was yet to be study. Thus, the result shows in Figure 3 has shown the potential of these distinctive bacteria strains to be implemented for decolourisation of azo dye in a form of consortia.

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According to previous study, the *E. casseliflavus* C1 bacteria has the ability to express an enzyme similar to azo reductase which contributes to the decolourisation of dye [14]. As the process of decolourisation occurred, the decolourised Amaranth dye will generates hydrazo intermediate, followed by a symmetric reduction cleavage to form aromatic amines such as 1-aminonapthelene-4-sulfonic acid and 1 aminonapthelene-2-hydroxy-3,6-disulfonic acid [12]. To transform these dye intermediate, it is found that the *C. freundii* A1 bacteria possess a flavin reductase enzyme in which capable to reduce azo bond and further degrading the dye intermediates [15]. Previously, it is also found that *C. freundii* A1 contain genes for degradation of benzoate, catechol, gentisate and protocatechuate [15]. Although lower level of decolourisation was achieved by *C. freundii* A1 as a pure culture, but it is found that *C. freundii* A1has the ability to further degrade intermediate compounds after decolourisation process through mineralisation processes [14, 16]. In addition, higher decolourisation was achieved by mixed culture due to the synergetic interaction that may have occurred among the bacterial strains which perhaps complement each other in their degradation capability. Although azo dye decolourisation rate was enhanced under the nutrient limiting condition, the presence of carbon source such as glucose and nutrient broth were still necessary for the microbial survival and decolourisation [14]**.** Therefore, it showed that *E. casseliflavus* C1 and *C.freundii* A1 bacteria consortia do not only able to decolourise the azo dye, but it able to further degrades the azo dye intermediates [13, 15].

3.2 Fourier Transform Infrared (FTIR) Spectrum Analysis for Dye Decolourisation

The determination of azo bond degradation was analysed by using Infrared spectroscopy (IR). Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed for the control (Figure 4a) and the decolorized sample (Figure 4b). The FTIR spectrum of the control dye displayed a peak at 3,311 cm^{-1} which corresponds to the intermolecular hydrogen bonding aromatic –OH and O-H stretching; a peak at 2,125.33 cm^{-1} for N-H stretching of amines; a peak at 1,507. 53 cm^{-1} for N=N stretching of azo group respectively. The decolourised sample spectrum showed a peak at 3,343.94 cm^{-1} for the intermolecular hydrogen bonding aromatic –OH and O-H stretching; a peak at 2,127.03 cm^{-1} for N-H stretching of amines. However, the peak located at 1,507. 53 cm^{-1} for N=N stretching of azo group disappeared after 120 minutes. Based on the previous study, this indicates that the azo bond was cleaved biologically in the anode chamber after the decolourisation occur [17].

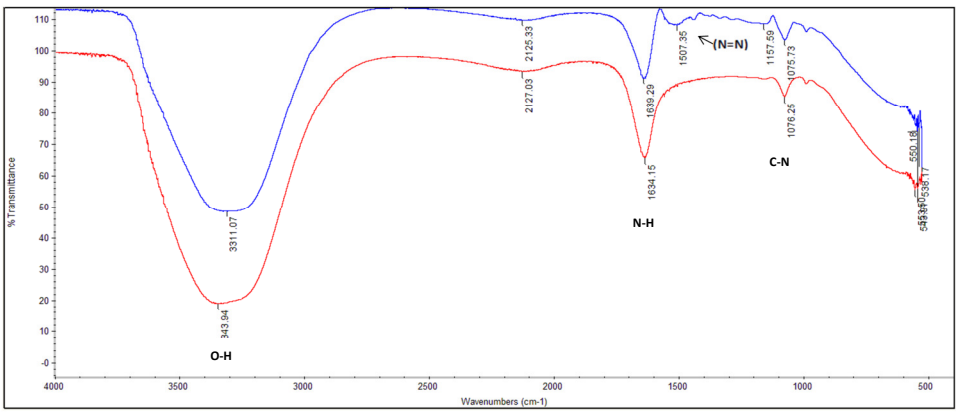

Fig. 4. FTIR spectrum of Amaranth dye during 0 minutes (a) and after 120 minutes (b) showing the absence of azo bond (-N=N-) after decolourisation process.

This was supported by the decrease in colour intensity of amaranth dye according to time in which from dark red colour to light yellowish as shown in Figure 5. These results were supported by previous study [12] which stated that the microbial degradation of azo dyes occured *via* the reduction of azo bonds.

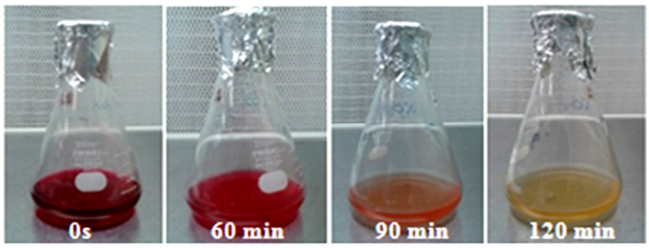

Fig. 5. Colour change of AR-27 dye over time at 29 ± 2ºC

3.3 Effects of Series and Parallel Connections in Voltage Productions

The bioelectricity generation by series and parallel connection of MFC was investigated through the decolourisation of AR-27 by *E. casseliflavus* C1 and *C.freundii* A1 in modified P5 medium. The effect of series and parallel connections in voltage generation by Acid Red 27 decolourisation in MFC was shown in Figure 6. Based on Figure 6, during the first 2 hours MFC operation, low voltage production was recorded during the azo dye decolourisation phase and the voltages generated starts to increase when complete decolourisation of AR 27 was achieved. This due to most of the electron generated through the co-substrates (glucose) oxidation of in the modified P5 medium was used by the bacteria consortia to reduce the azo linkage (-N=N-) with the presences of azo reductase enzyme [13]. As the decolourisation of AR 27 was achieved, it is expected that the increased in voltage generation in MFC was caused by the degradation of azo dye intermediates and autooxidation reaction [13]. For the bioelectricity study, Figure 6 demonstrated that the usage of series and parallel connections in MFC has the ability to generate higher voltages or currents respectively. The performance of a single unit MFC was improved when several individual cells was connected together in series or parallel modes [18]. Therefore, connection of three individual units MFC was used to increase bioelectricity generation in the form of stacked MFC.

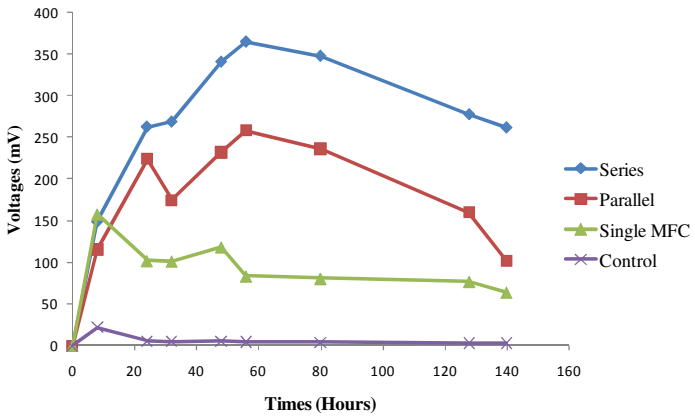

Fig. 6. Effects of series and parallel connections in voltage generations in open circuit voltage (OCV).

Three cathodic and three anodic chambers were connected in parallel and series to each other to make a stacked MFC. Based on Figure 6, the maximum voltage generated by the single unit MFC in OCV was the lowest which were 118.3 mV within 12 hours operational time and the amount of voltages production continue to decrease after the next 24 hours. In contrast, the maximum generated OCV in series and parallel modes were 365 mV and 258 mV, respectively. The maximum voltage productions for series connected MFC and parallel connected MFC was recorded within 60 hours operational time. This result showed that combining proper numbers of MFC in parallel or series improved the current or voltage obtained [19]. Comparing results produced between series and parallel connections in Figure 6 revealed that voltage generated by series connections were two folds higher than that of parallel connections. Thus, indicates that connecting several fuel cells in series would increase the voltages, while one common current flow through all fuel cells. In case several power sources are connected in parallel, the voltage averages and the currents are improved [19].

3.4 Current Density and Power Density Curves

The current density and power density curve were obtained from the voltage produced by the close circuit voltage (CCV). The voltage profiles of the 3 individual unit MFCs are shown in Figure 7a for series connections and Figure 7b for parallel connections. Based on these results, it shows that the MFCs connected in series connections produced the highest CCV of 244 mV with 100 kΩ external load. From the polarisation curve shown in Figure 7c and 7d, the maximum power output achieved for series connections was 213µW/cm^2. In comparison the power output from the parallel connection was 54.4µW/cm^2. This shows that power density of series connections was approximately four times higher than that of parallel connections.

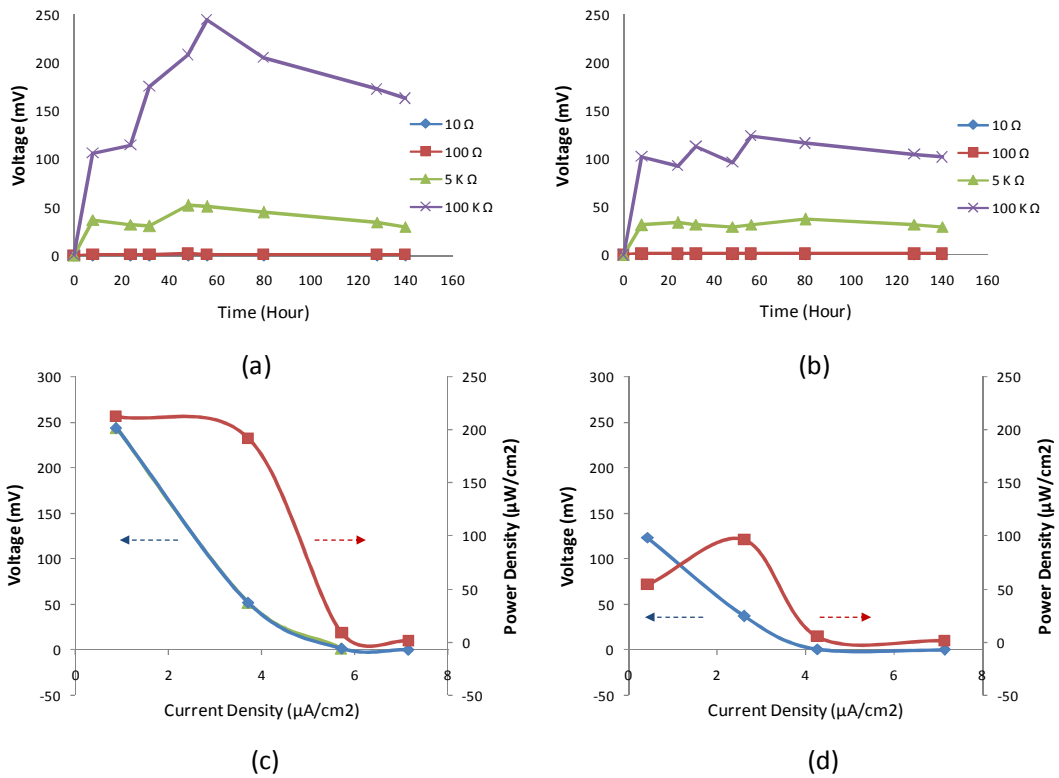

Fig. 7. Effects of external load towards series (a) and parallel (b) connections in voltage generations in CCV. Polarisation curves for series (c) and parallel (d) connections.

Although, the three MFC units used the same inoculum and shared the same analyte solution and were exposed to the same air flow and temperature, the performance differences are believed to be due to the electrode variances during manufacturing process, such as uneven application of the cathode diffusion layer caused inefficient mass transfer [20]. In term of the current density, when the units were connected in series the maximum current density of 8.71×10^{-4} mA/cm^2 was obtained at 10 Ω external resistance. This value decreased to 4.41×10^{-4} mA/cm^2 when connected in parallel. The current density output for series connections were 1.98 fold higher compared to that of parallel connections.

Figure 7(c) and Figure 7(d) show that for the resistances of 10 Ω, 100 Ω, 5 k Ω and 100 k Ω there was a simultaneous increase between the current density and power density. Previous study reported that the power density of the fuel cell will decrease with the increasing of current density [21]. Similar patterned was noted in Figure 7(a) and 7(b) that showed an increase in the power density with an increase in current density before which the power density began to decrease with a further increase in current density. The current generation showed a decreasing trend with an increase in resistance, which is consistent with the reported literature [22] and indicated typical fuel cell behaviour. This shows that both single unit MFC and stack MFC was in line with this principle when higher external load was introduced, These polarization curves show that the MFCs connected in series and parallel, worked, respectively, at an average current and voltage determined by the performance of the individual MFCs. Drawing excessive current from a fuel cell at a rate higher than its fuel delivery supports, leads to an increase of the anode potential and subsequent cell reversal [23]. Fuel starvation, i.e., an inadequate supply of fuel, is a major cause of cell reversal and can occur during a sudden change of fuel demand such as during start-up or a change of the load [23].

3.5 Current Density and Power Density Curves

A comparative overlay of cyclic voltammogram throughout the azo dye decolourisation is presented in Figure 8. Based on the figure, the sterile modified P5 medium containing amaranth dye were used as control has shown the presence of high current peak for both oxidation and reduction. This is due to Azo dyes showed similar electrochemical characteristics in the cyclic voltammograms [14]. All compounds exhibited well-defined reduction peak, Er, and oxidation peak, Eo [24]. When the bacteria were introduced, the magnitude of the oxidation and reduction peak was decreased with the time as shown in Figure 8 due to the degradation of amaranth dye in which subsequently reducing the concentration of dye in the medium.

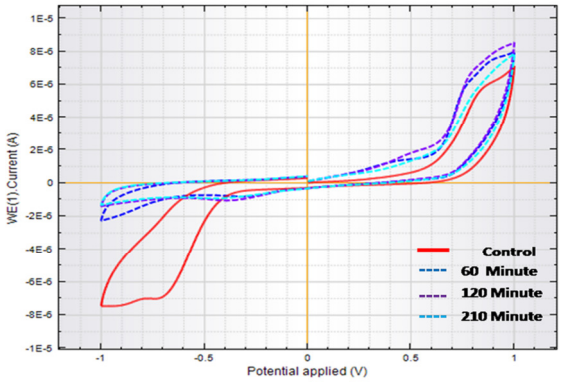

Fig. 8. Cyclic voltammogram throughout the azo dye decolourisation by *C. freundii* A1 and *E. casseliflavus* C1 bacteria consortia.

The CV has confirmed that the AR27 dye was reduced lower reduction potential of -0.8 V and similar result were reported by previous literature [25]. Based on the results, the difference between the oxidation reductions peaks can be observed after complete decolourisation was achieved. Thus, it is assumed that throughout the decolourisation process, both the reduction peak was decreased as the azo degrading bacteria (*E. casseliflavus* C1 and *C.freundii* A1) are broken down the azo bond during the decolourisation and decreased in co-substrates inside the modified P5 media.

3.6 Scanning Electron Microscope (SEM) Imaging

In MFC, all biochemical reaction will be trigger in the anode due to the presences of microorganisms community that will be utilise the organic or inorganic component and transmit electron specifically to the anode surface. All this reaction will be ended at the cathode that participates in the final electron reaction. Scanning Electron Microscope (SEM) analysis was applied to investigate the morphological of the anode surface after MFC operation. Here, graphite felt was used as the electrode for the anode and cathode chamber throughout the MFC operation. Initially, the graphite felt electrode before the MFC operation was used as control and the SEM imaging showed a fibrous regular form of the electrode in Figure 9(a) without any bacterial attachment onto its surface.

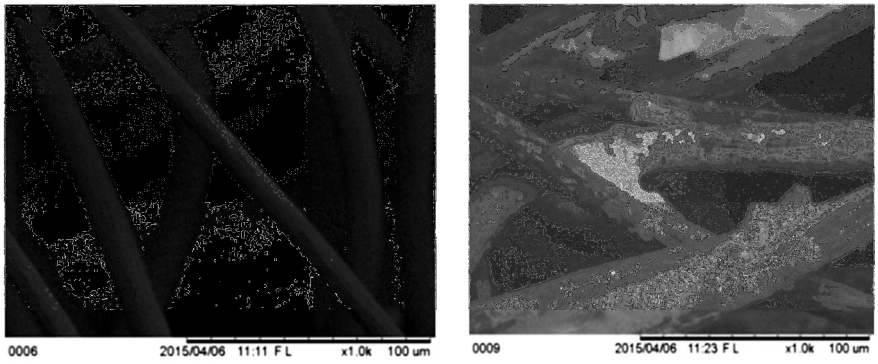

Fig. 9. Scanning electron micrograph on carbon cloth before (a) and after of MFC operation (b)

However, the SEM analysis shows random bacterial attachment was observed on the anode surface with the presence of some amount of particulate after the MFC operation as shown in Figure 9(b). Moreover, the result obtained was also consistent with previous study which had confirmed that the bacteria did not spread uniformly but attached in random sections of the material as shown in Figure 9 (b) [26, 27]. This might due to the intrinsic preference of the bacteria to colonize the less hydrophobic surface or less hydrophobic part of an electrode surface. Moreover, previous literature stated that electron transfer by bacteria to electrode can be done by direct transfer of electron, involved the use of mediator or the formation of nanowire (pilli) by the bacteria [28]. Figure 9(b) shows that the bacteria attachment on the anode surface indicates the electron was transmit directly from the bacteria to the electrode due to the absence of nanowire or the used of mediator during the MFC operation for the bioelectricity generation.

4. Conclusion

The used of bacterial consortium (*C. freundii* A1 and *E. casseliflavus* C1) shows the highest and fastest azo dye decolourisation efficiency. Moreover, the MFC that had been connected in series and parallel has also achieved more than 90% decolourisation efficiency with this bacteria combination. However, series mode showed the highest output of voltage in OCV set up with 365 mV generated. While, In CCV set up the amount of voltage generated was 244 mV with the power density and current density of 212.629µW/cm^2 and 0.871µA/cm^2 respectively when 100 kΩ resistances introduced. Thus, this indicates that the series connections have the capabilities to increases the amount of voltages generated by MFC.

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