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Bioelectricity generation based on Acid Red 27 decolourization in microbial fuel cell

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ABSTRACT

Microbial Fuel Cell (MFC) is a device that generates electricity from the metabolism of bacteria simultaneously treats wastewater by decolourizing the azo dye in wastewater. In this work, the effect of different external loads and bacterial loads were examined. The maximum open circuit voltage generated was 390 mV by using 7 consortia of bacteria while the maximum current generated was 50 µA using 10 Ω resistor. 97% decolourization efficiency of 0.1 g/L of azo dye was achieved after 5 days of operation. Besides, the maximum current density and power density achieved were 17.9 µA/cm^2 and 460 µW/cm^2 respectively. Polarization curve was plotted and Scanning Electron Microscope was applied to visualize the bacterial community attachment onto the graphite felt electrode. Cyclic voltammetry was applied to study the redox properties of the Azo dye using microorganisms in MFC. Overall, these 7 bacterial strains used in this work showed the capability in decolourizing the Azo dye simultaneously producing electricity in MFC.

1. Introduction

Energy crisis and water pollution are the two important issues nowadays. Alternative method is necessary to re-place and reduce the dependency on fossil fuels which will be run out few years later for electricity generation [1]. Besides, effluents especially from textile industries discharged into the environment contain various dyes that are not eco-friendly. Microbial fuel cell (MFC) has emerged as a bio-electrochemical system to treat wastewater simultaneously generates electricity from wastewater treatment pro-cess [2]. In this research, parameters that affected the performance of the electricity generation and decolourization of azo dyes included bacterial load and external load, were optimized to maximize the electric cur-rent production and simultaneous decolourization of

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azo dye. Acid Red (AR) 27, the azo dye present in the wastewater sample was degraded based on the visual observation of colour change from red to colourless by breaking the azo bond. Biological approach is chosen to treat wastewater instead of other approaches like physio-chemical methods which involve the process of chemical precipitation, coagulation–flocculation, ion exchange and membrane filtration [3] due to the costly operation and complicated process is needed. On the other hand, biological approach is more environment protective and economically feasible in wastewater treatment process. This research aimed to grow and culture the electroactive bacterial strains in the microbial fuel cell to determine parameters that affect the MFC performance and the surface morphology of electrodes was examined using Scanning Electron Microscope (SEM). These bacterial strains are expected to produce outstanding performance in bioelectricity generation compared to the previous bacteria studied by other researchers so that it can re-place the non-renewable source, fossil fuels to sustain the high demand of the world [4].

2. Materials and method

2.1. Culture and medium

Seven bacterial strains namely B1, POS, POS M2, PCO oil, M2, F2b, and 7a were used in this study and they were obtained from the Microbiology lab collection, UTM in nutrient broth. Chemically Defined Medium (CDM) was synthetic medium that used in determining decolourization efficiency of bacteria against AR 27 and medium in anodic chamber of MFC. CDM contains K_2HPO_4 (7 g/L), KH_2PO_4 (2 g/L), $MgSO_4.7H_2O$ (0.1 g/L), $CaCl_2$ (0.02 g/L), $(NH_4)_2SO_4$ (1 g/L), Glycerol (1 g/L), AR 27 (0.1 g/L) which has been modified with the components of single pure azo dye, Acid Red as the carbon source [5]. To prepare the inoculum of bacteria, 1 mL of medium with bacterial strains was pipetted and centrifuged at 10,000 rpm for 3 minutes. Supernatant was discarded and the pellet was suspended with 1 mL of distilled water. The suspended pellet was then poured into CDM. The culture was then then incubated in incubator shaker at 37°C overnight. Optical density was read using UV-Vis spectrophotometer at 600nm to ensure the bacterial strains was grown above OD 0.6 prior to inoculation.

2.2. Decolourization of Azo dye

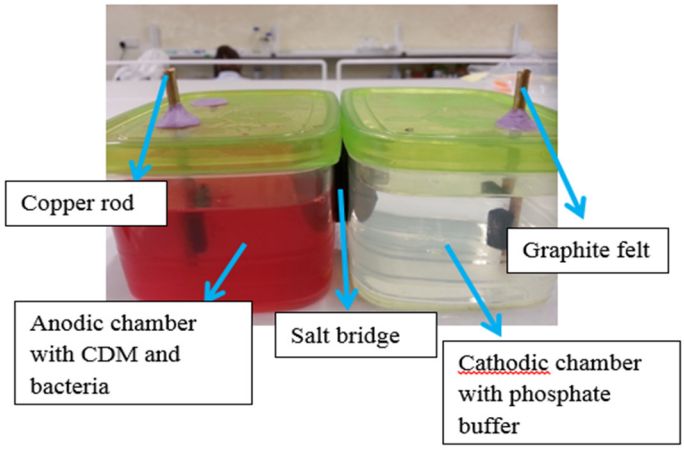
Different combinations of bacterial strains were studied to determine the best bacterial consortium in decolourizing AR 27. 1 mL of bacterial strains was pipetted from the working stock and centrifuged at 10,000 rpm for 3 minutes to obtain the pellet. The pellet was suspended in 1 mL of distilled water and poured into 100 mL conical flasks with 3/4 volume of CDM to reduce oxygen diffusion into the medium. The conical flasks with different combinations of bacteria were then incubated in incubator at 37°C. Decolourization occurs when there was a decrease in absorbance at a wavelength of 521 nm. The decolourization efficiency of combination bacterial strains was observed and the optical density reading was taken every day using UV-Vis spectrophotometer. 1 mL of the medium out and centrifuged at 10000 rpm for 3 minutes. Optical density was read using supernatant with distilled water was used as blank. Decolourization efficiency or colour removal was determined by the Eq. 1 [5]:

$$\text{Decolourization efficiency (\%)} = [(Ai-Af)] / Ai \times 100\% \quad (1)$$

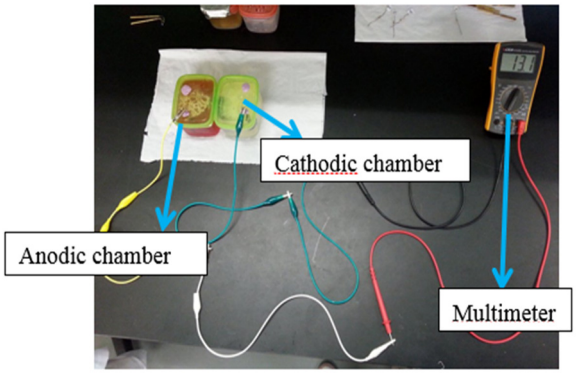
Where Ai refers to the initial absorbance of dye prior to operation, Af to the final absorbance at any time.

2.3. Microbial fuel cell configuration and operation

A two compartment Microbial Fuel Cell was made up of two chambers of total 140 mL working volume (6cm × 8cm × 4cm) connected by salt bridge. In anodic chamber, it was filled with Chemically Defined Medium (CDM) with the addition of azo dye and cathodic chamber was filled with phosphate buffer of pH 7 while 1 cm × 1 cm × 0.2 cm of graphite felt was attached to the copper rod as the electrode. External wires were connected to the anode and cathode of the MFC to the multimeter using crocodile clips. This MFC was run under room temperature and the voltage generated every day was measured. 25% and 15% of bacterial load were prepared to determine the effect of bacterial load on voltage generation and different resistance values of 10 Ω, 100 Ω, 5 KΩ, and 100 KΩ were applied to determine the effect of external loads on voltage generation in MFC.



(a)



(b)

Fig. 1. (a) Configuration of a Microbial Fuel Cell (MFC), (b) Configuration of a microbial fuel cell without resistor (open circuit voltage).

Maximum voltage generated with resistance from each set of MFC was used to determine the current density and power density of the system. The current (I) and power (P) density were normalized to the anode [6] and polarization curve was constructed.

2.4. Cyclic voltammetry

For the qualitative analysis, potentiostat was used to determine the redox properties of MFC through cyclic voltammetry before and after decolourization. Two media were prepared. The first medium acted as the control consists of 40 mL of medium with 0.1 g/L azo dye without any bacterial strains (before decolourization of azo dye). The second medium was 40 mL medium with 0.1 g/L dye and with 7 bacterial strains (after decolourization of azo dye). Two different scan rates selected were 10 mV/s and 30 mV/s, respectively. The peaks obtained before and after the decolourization of azo dye were compared to determine the redox behaviour of Amaranth and electrochemical reversibility of the system.

2.5. Scanning electron microscope

The Scanning Electron Microscope (SEM) analysis was carried out to examine the surface morphology of the electrode, before and after decolourization of amaranth dye. The graphite felt (1 cm × 1 cm × 0.2 cm) originally attached on copper rod was removed from the anode of MFC and placed onto the aluminium foil. It was then put inside a furnace for drying (water removing) at 70°C for overnight. The dried samples were then collected and sent for Scanning Electron Microscope measurement.

3. Results and discussion

3.1. Decolourization efficiency of different mixtures of bacterial strains

The effect of mixtures of bacterial strains in decolourizing AR 27 was determined using different combinations of bacterial strains.

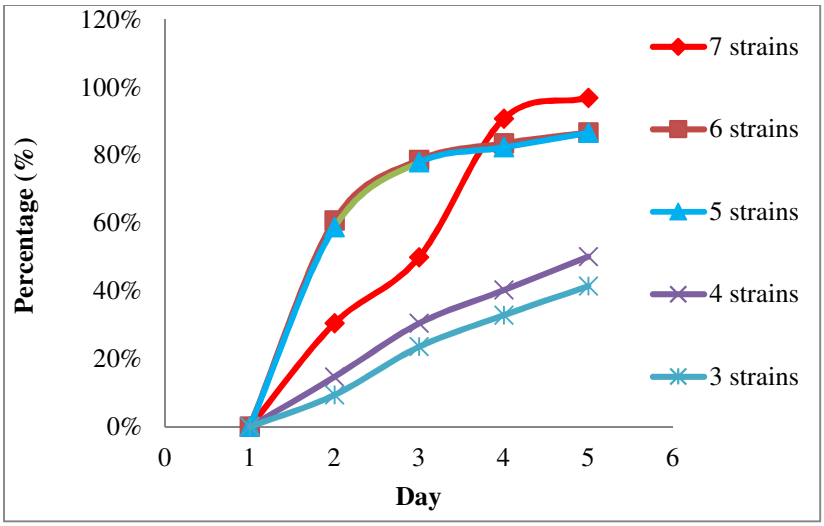
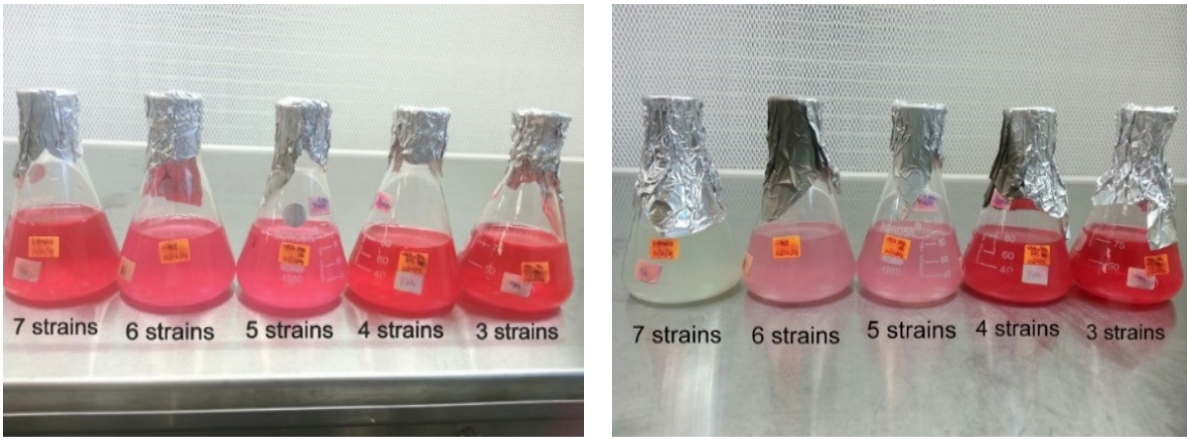


Fig. 2. Decolourization efficiency of different combination of bacterial strains.



(a) **(b)**

Fig. 3. Decolourization of azo dye of different combinations of bacterial strains before (a) and after (b) 5 days.

From Fig. 2, the highest efficiency in decolourizing process of azo dye was the combination of 7 strains of bacterial strains which contains B1, POS, POS M2, M2, PCO oil, F2b, 7a. 97% of azo degradation was achieved after 5 days. This might be due to the presence of bacteria in those 7 type of bacterial strains which could degrade the azo dye aggressively. This bacterial strains might be 'M2' which was excluded from the 6 combinations of bacterial strains. Therefore, bacterial strain M2 is important in decolourization of azo dye as other combinations of bacterial strains (5, 4, and 3 strains) did not contain M2 in the medium as well. Besides, the decolourization rate of azo dye by 7 bacterial strains was slower at the first 4 days compared to the combinations of 6 and 5 bacterial strains. This might be due to the presence of bacterial strain M2 that affects the degradation process. It might due to the behaviour of M2 in the medium which took longer time to get along with other bacteria in the medium. The interaction of M2 with other bacteria and adaptation in the CDM might be poor which in turn affected the overall decolourization process. It started to perform to degrade azo dye intensively in the presence of other 6 bacterial strains after 5 days resulting in colour change from red to colourless.

On the other hand, the combination of 6 and 5 strains of bacteria also reduced much azo bonds in the CDM as it showed a pinkish colour of CDM as shown in Fig. 3. However, the efficiency of decolourizing azo dye of bacteria with combination 6 strains and 5 strains were the same. Both combinations have degraded 87% of the azo dye. Based on the results obtained, the more combination of bacterial strains, the faster the process of degradation of azo dye. This might signify a complementary interaction among various bacterial strains [7]. As the result, 7 strains of bacterial isolates were chosen to be inoculated into MFC to determine the parameters that affect the performance of MFC on electricity generation. To make sure that the decolourization process is completed, minimum 5 days was needed to incubate the medium which azo dye with bacteria.

3.2. Effect of bacterial load on voltage generation in MFC

Different amount of bacterial strains (25% and 15% of the total volume) were used to determine the relationship between bacterial load and the voltage generation in MFC. Open circuit voltage (OCV) is the measure of maximum voltage generated without any resistance applied. As the result, OCV showed the highest reading of voltage in each set of MFC as shown in Fig. 4.

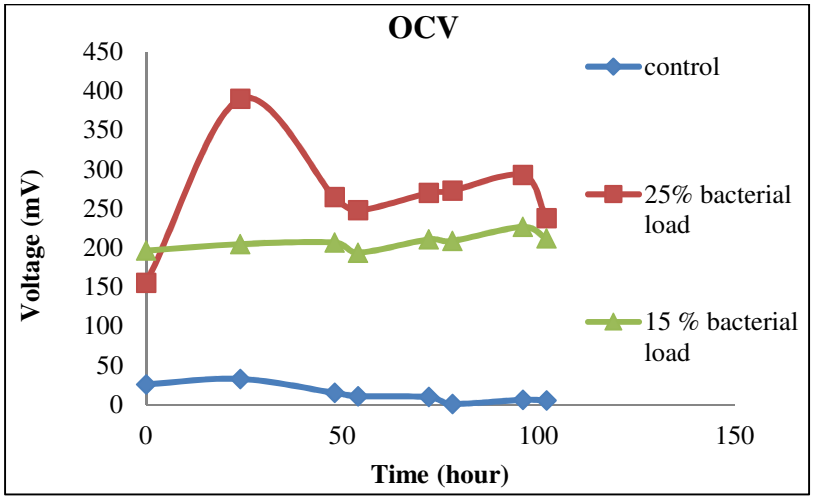


Fig. 4. Effect of bacterial load on voltage generation in Microbial Fuel Cell without applying any resistance (open circuit voltage).

As shown in Fig. 4, the higher the bacterial load in the medium, the higher the voltage generated. There was around 1.5 times higher in voltage generation when the concentration of bacteria was increased by 10%. This might be due to fact that higher bacterial load has higher bacterial activity that is responsible for the breaking down azo bond in the azo dye, which means the higher number of electrons will be produced from this reaction. Higher amount of electrons will be released to anode in a faster rate as there was high substrate (azo dye) conversion rate which in turn generated higher voltage in the MFC [8].

3.3. Effect of external load on voltage generation in MFC

Different resistor values were used in this work to determine the effect of external loads on voltage generation in MFC. Resistor values used were 10 Ω, 100 Ω, 5 KΩ, and 100 KΩ.

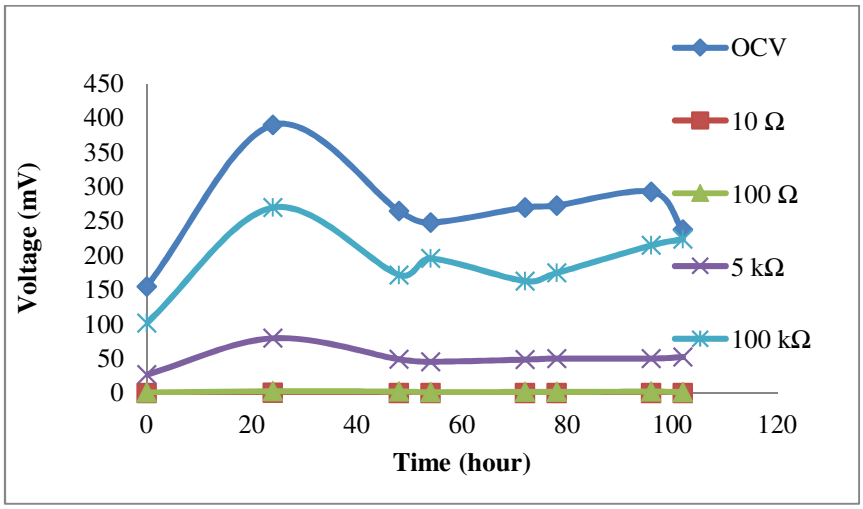


Fig. 5. Effect of external load on voltage generation in MFC set 1 with 25% bacterial load

According to [9], external resistance manipulates the ratio between cell voltage and current generation implies that the higher external resistance results in higher cell voltage and vice versa. As shown in the Fig. 5**,** increasing resistor values from 10 Ω to 100 Ω, 5 kΩ, and 100 kΩ, resulted in higher

voltage generation. However, there was not much difference in terms of voltage generation between the 10 Ω and 100 Ω as the resistance gap is quite small (10 times). There was an elevated voltage generation which about 4 times higher when the resistance increased from 5 kΩ to 100 kΩ.

3.4. Current density and power density

The maximum current density and power density generated from the MFC set 1 which contains 25% bacterial load were 17.9 μA/cm^2 and 0.46 mW/cm^2 respectively as shown in Fig. 6.

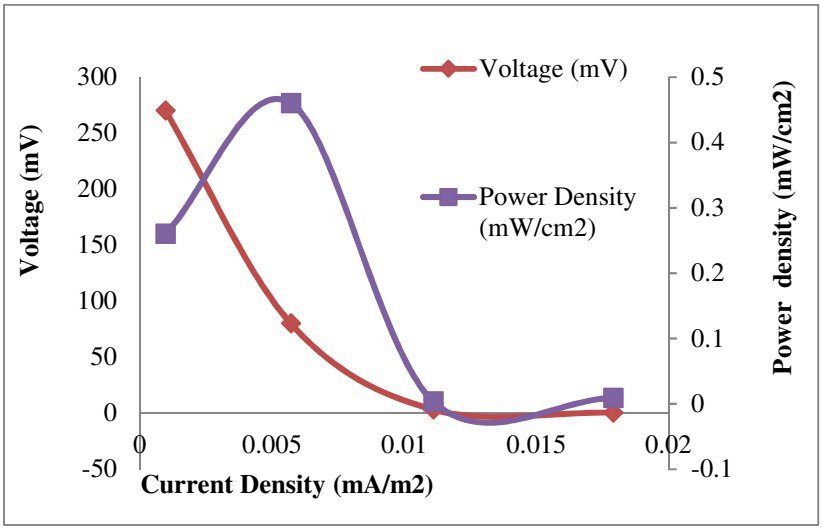
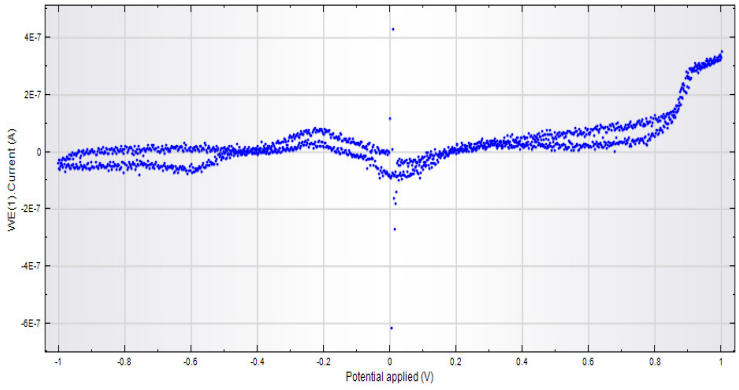


Fig. 6. Polarization curve of MFC set 1

The power density obtained in this experiment was moderately good as compared to previous research performed by [10] in MFC using electricity-producing bacterial communities, which produced power density of 0.431 mW/cm^2. On the other hand, previous research by [11] showed the current density obtained were 12.5 μA/cm^2 from wastewater treatment when using a single chamber microbial fuel cell. The polarization curve shows the relationship between voltage, current density and power density. As stated by Ohm's law, the higher the current density resulted in the lower the voltage and vice versa. On the other hand, the increase of current density gave rise to the increase of power density. However, until a certain level, there was a decrease in power density when the current density was further increased. This indicated a typical fuel cell behaviour as reported by [12]. This sharp drop of power density at higher current density might be due to the power overshoot according to [13] and additional tests are required to find out this phenomenon.



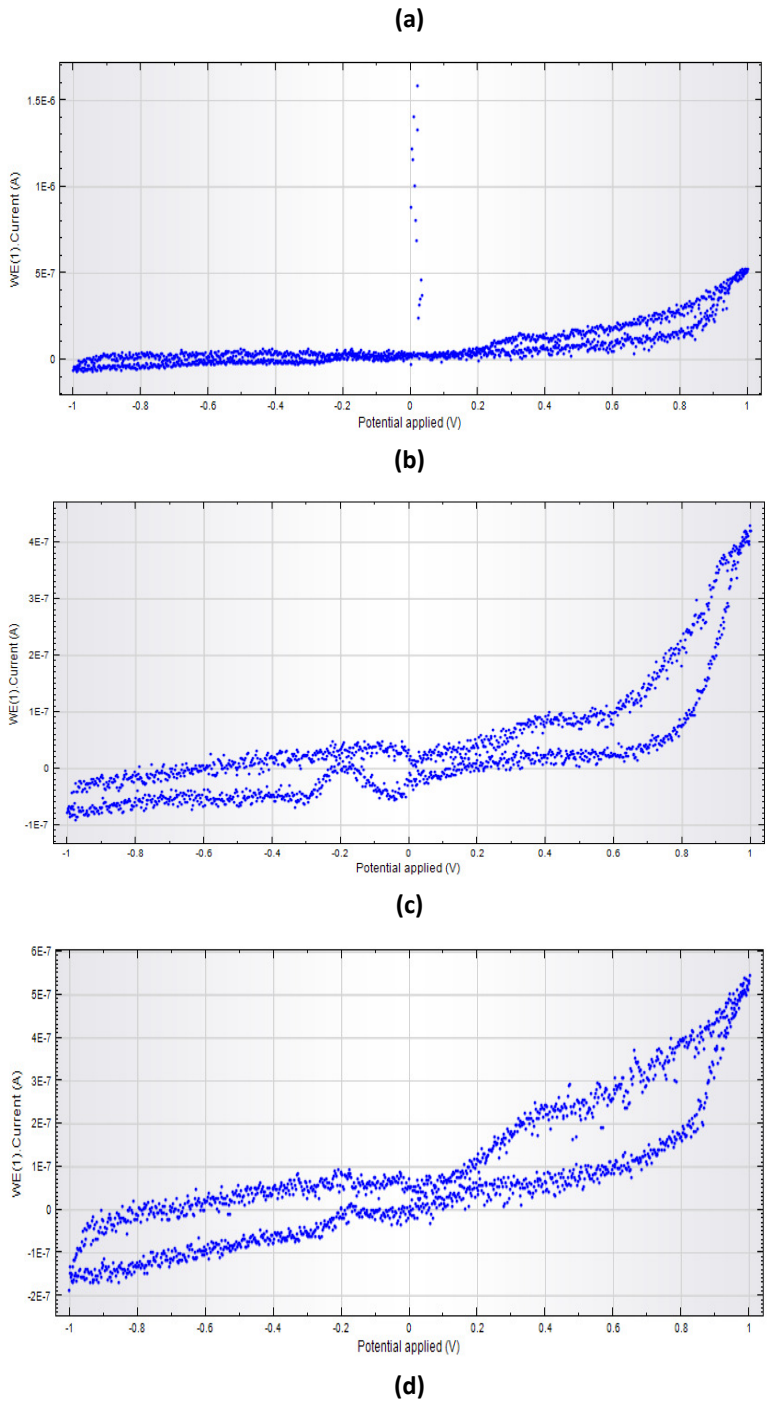


Fig. 7. Cyclic voltammogram of 0.1 g/L of azo dye a) scan rate 10 mV/s before decolourization, b) scan rate 10 mV/s after decolourization, c) scan rate 30 mV/s before decolourization, d) scan rate 30 mV/s after decolourization.

3.5. Cyclic voltammetry

Cyclic voltammetry is commonly used to study the electroactive species in the medium [14]. Media with azo dye but without bacteria was analysed following by media with bacteria after the decolourization of azo dye to compare the changes happened to the azo dye before and after the decolourization as shown in Fig. 7. Different scan rates of 0.01 v/s and 0.03 v/s were used. Those peaks which appeared above 0 V from x-axis indicate the reduction peak while peaks below 0 V from

x-axis indicate oxidation peak [14]. Based on the results obtained, there were few peaks appeared before the decolourization (Fig. 7a) while there showed no peak at all after the decolourization of azo dye (Fig. 7b). The downward peak occurred at -600 mv (Fig. 7a) may indicate the dye removal as stated by [15]. In this experiment, bacteria might have degraded the azo dye as its part of metabolism where azo dye has been taken up as their substrates. Besides that, an upward peak that occurred at around -200 mV (Fig. 7a) may be due to the reduction of the products resulting from the events accounting for peak at -600 mV [14]. From Fig. 7c and 7d, it showed that there was no significant difference of the peaks before and after the decolourization of azo dye using higher scan rate of 30 mV/s except a peak which appeared at -200 mV (Fig. 7c). Therefore, a suitable scan rate should be used to give obvious reduction and oxidation peak. In addition, cyclic voltammetry can determine the electrochemical reversibility of the system by measuring the oxidation and reduction peak potential separation and frequency [16]. For an electrochemical reversible system, the separation between oxidation peak and reduction should be 60mV [17]. Unfortunately, the peaks of oxidation and reduction were no obviously to be determined from the results obtained.

3.6. Characterization of biofilm formation at anode

Characterization of biofilm formation on electrode was conducted by using Scanning Electron Microscope (SEM). Different magnification powers include 50 times, 100 times, 500 times, 2.5k times, 6k times were applied to investigate and observe the biofilm formation on electrode. The bare graphite (no modification) was used as the control set in comparison to the modified one as shown in Fig. 8a. Fig. 8b and 8c shows the bacterial attachment on graphite felt.

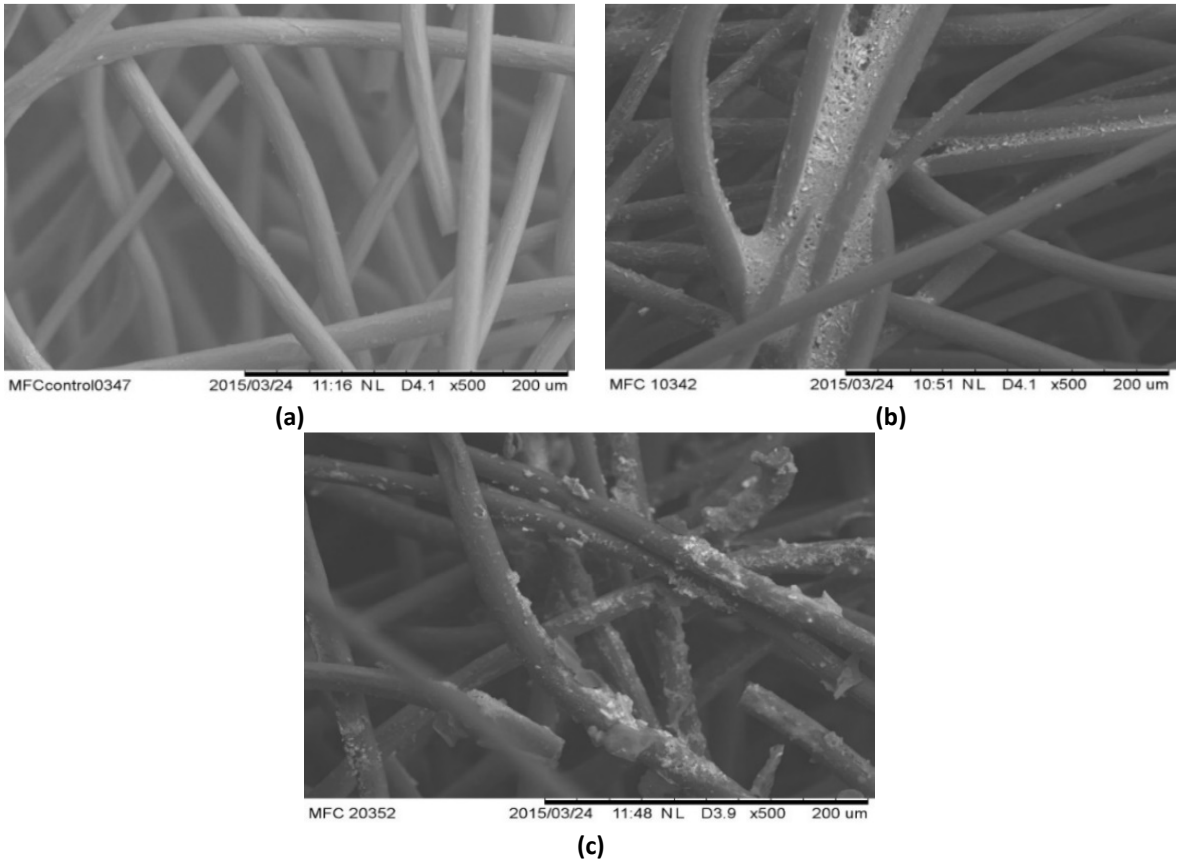


Fig. 8. Scanning electron micrograph of graphite felt on anode of a) control set of MFC b) MFC set 1 c) MFC set 2.

Bacterial attachment was observed at the anode surface in the MFC set 1 and 2 (Fig. 8b and 8c) but not in the control set. This is due to bacteria attached to anode where the oxidation is occurred in MFC. Bacteria releases electrons produced from cell respiration to anode when catalyses the oxidation of the media, CDM (reduced substrates) [10]. The electrons then flow through external circuit to cathode (counter electrode) to generate current [10]. Based on Fig. 8b and 8c, there were different morphologies of bacterial attachment being observed although they contained the same combination of bacterial strains. Bacterial attachment in MFC set 1 is in biofilm conformation while bacterial attachment in MFC set 2 was in stack form with no biofilm formation. Biofilm is the well-organized, cooperating communities of microorganisms attached to a surface [18]. The formation of biofilm could be affected by surface roughness (substratum effect), conditioning film (coated with polymers from the medium), hydrodynamics (turbulent or laminar flow), physio-chemical characteristics of aqueous medium (pH, ionic strength, temperature) and others [18]. In this work, MFC set 1 contained higher amount of bacteria load which led to the biofilm formation and resulted in higher voltage reading compared to MFC set 2. In MFC set 2, which there was no biofilm formation observed which might be related to the lower voltage generated compared to that of MFC set 1. This may probably due to the probability that bacterial growth and activities increase by the incorporation of surfaces to which microbes can attach which is also known as the Bottle effect [18].

4. Conclusion

In conclusion, the combinations of 7 bacteria strains gave the highest decolourization efficiency of 97% after 5 days of operation. The highest OCV generated was 390 mV by using 7 consortia of bacteria while the maximum current generated was 50 µA using 10 Ω resistor. Besides, the maximum current density and power density achieved were 17.9 µA/cm^2 and 460 µW/cm^2 respectively from MFC set 1 with 25% of bacteria load. Biofilm formation was visualized on the anode of MFC set 1 using SEM. Besides, the reduction of azo dye was observed from the reduction produced after performing cyclic voltammetry. In overall, MFC shows that it is a promising way to decolourize azo dye in wastewater and produce electricity at the same time.

Acknowledgements

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References

[1] Hayder, G., Puniyarasen, P., "Identification and Evaluation of Wastes from Biodiesel Production Process". Journal of Advanced Research in Applied Sciences and Engineering Technology. Vol. 3, No.1, (2016): 21-29.

[2] Singh, D., Pratap, D., Baranwal, Y., Kumar, B., and Chaudhary, R. "Microbial Fuel Cells: A Green Technology for Power Generation". Annals of Biological Research. 1(3): (2010). 128-138.

[3] Kurniawan, T. A., Chan, G., Lo, W.-H., and Babel, S. "Physico–Chemical Treatment Techniques for Wastewater Laden with Heavy Metals". Chemical Engineering Journal. 118(1): (2006). 83-98.

[4] Baranitharan, E., Khan, MR., Yousuf, A., Teo, W.F.A., Tan, G.Y.A., Cheng, C.K. "Enhanced power generation using controlled inoculum from palm oil mill effluent fed microbial fuel cell". Science Direct. Fuel 143: (2015). 72–79.

[5] Nawahwi, M., Ibrahim, Z., and Yahya, A. "Degradation of the Azo Dye Reactive Red 195 by Paenibacillus spp. R2". Journal of Bioremediation and Biodegradation 4: (2013). 174.

[6] Min, B., Cheng, S., and Logan, B. E. "Electricity Generation using Membrane and Salt Bridge Microbial Fuel Cells". Water Research. 39(9): (2005). 1675-1686.

[7] Jadhav, J., Kalyani, D., Telke, A., Phugare, S., and Govindwar, S. "Evaluation of the Efficacy of a Bacterial Consortium for the Removal of Color, Reduction of Heavy Metals, and Toxicity from Textile Dye Effluent". Bioresource Technology. 101(1): (2010). 165-173.

[8] Rabaey, K., and Verstraete, W. "Microbial Fuel Cells: Novel Biotechnology for Energy Generation". TRENDS in Biotechnology. 23(6): (2005). 291-298.
[9] Aelterman, P., Versichele, M., Marzorati, M., Boon, N., and Verstraete, W. "Loading Rate and External Resistance Control the Electricity Generation of Microbial Fuel Cells with Different Three-dimensional anodes". Bioresource Technology. 99(18), (2008): 8895-8902.
[10] Logan, B. E., and Regan, J. M. "Microbial Fuel Cells-Challenges and Applications". Environmental Science and Technology. 40(17): (2006). 5172-5180.
[11] Liu, H., Ramnarayanan, R., and Logan, B. E. "Production of Electricity During Wastewater Treatment Using A Single Chamber Microbial Fuel Cell". Environmental Science and Technology. 38(7): (2004). 2281-2285.
[12] Mohan, S. V., Saravanan, R., Raghavulu, S. V., Mohanakrishna, G., and Sarma, P. "Bioelectricity Production from Wastewater Treatment in Dual Chambered Microbial Fuel Cell (MFC) using Selectively Enriched Mixed Microflora: Effect of Catholyte". Bioresource Technology. 99(3): (2008). 596-603.
[13] Watson, V. J., and Logan, B. E. "Analysis of Polarization Methods for Elimination of Power Overshoot in Microbial Fuel Cells". Electrochemistry Communications. 13(1): (2011). 54-56.
[14] Kissinger, P. T., and Heineman, W. R. "Cyclic Voltammetry. Journal of Chemical Education". 60(9): (1983). 702.
[15] Fan, L., Zhou, Y., Yang, W., Chen, G., and Yang, F. "Electrochemical Degradation of Aqueous Solution of Amaranth Azo Dye on ACF Under Potentiostatic Model. Dyes and Pigments". 76(2): (2008). 440-446.
[16] Nicholson, R. S. "Theory and Application of Cyclic Voltammetry for Measurement of Electrode Reaction Kinetics". Analytical Chemistry. 37(11): (1965). 1351-1355.
[17] Cooper, S. R., McArdle, J. V., and Raymond, K. N. "Siderophore Electrochemistry: Relation to Intracellular Iron Release Mechanism". Proceedings of the National Academy of Sciences. 75(8): (1978): 3551-3554.
[18] Kokare, C., Chakraborty, S., Khopade, A., and Mahadik, K. (2009). "Biofilm: Importance and Applications". Indian Journal of Biotechnology. 8(2): (2009). 159-168.