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Decolorization of Azo Dye AR27 and bioelectricity generation in microbial fuel cell

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ABSTRACT

In this study, we investigate the ability of the bacterial isolates from an Iraqi oil reservoir, namely POS and PCO Oil to decolorize commercially used model azo dye Acid Red-27(AR-27). The effects of inoculation volume and glycerol concentrations were optimized to develop an economically feasible decolourization process. The isolates were able to decolourize azo dye (AR27) at the highest decolorization efficiency of 98% in 10 mL bacterial solution consisted of POS and PCO Oil and in the presence of 6.34 g/L glycerol. An optimized MFC using this bacterial consortium (POS + PCO Oil) and graphite rod electrodes produced a maximum open circuit voltage (OCV) of 175 mV, in the presence of potassium ferricyanide as the electron acceptor at the cathode. The maximum current density of 1.7 μA/cm² and power density of 59.3 μW/cm² were achieved when an external load of 5 kΩ was applied. Morphological analysis was performed using Scanning Electron Microscope (SEM) to prove the bacterial attachment onto the anode surface (graphite rod) in the MFC operation. This work proposed that the bacterial strains POS and PCO Oil possess the ability to decolorize Azo dye AR27 and generate electricity in the absence of nitrogen source.

Keywords:
Microbial fuel cell, Bacterial consortium, Azo dye decolourization, Power density, Current density, Ferricyanide, Glycerol concentration

1. Introduction

Nowadays, the world is facing serious environmental threats such as fossil fuel depletion, environmental pollution, water, energy and other resources shortage. Among these problems, intensive efforts have been developed towards the more sustainable treatment and utilization of wastewater [1]. Azo dyes found in the textile wastewater gives bright and high intensity colors, but not to forget the carcinogenic effect of azo dyes onto the environment. In 1970, Russian studies

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showed that AR27 was carcinogen [2]. In 1994 an estimation of approximately million tons of dyes were produced around the world in which more than 50% were azo dyes [3]. Azo dyes are characterized by the presence of azo groups (-N=N-) which most of them being xenobiotics; a substances foreign to entire biological systems. In fact, not all dyes molecules are bound to the fabric during the dyeing process, some of them end up in the industrial wastewater and potentially in the environment. About 2% and as much as 50% of the dyes used ended up in the wastewater, based on their class [4]. Many dyes are visible in water at concentrations as low as 1 mg/L. Textile processing wastewaters with dye contents in the range of 10–200 mg/L are highly colored. Some of the dyes and their degradation products are carcinogenic in nature [5].

Currently, a wide range of physicochemical methods to remove dyes from textile wastewater has been applied. The most commonly used technique is coagulation or flocculation processes [5]. This method requires the usage of chemicals in significant quantities and it will produce an amount of sludge which requires further handling and disposal. Other than that is adsorption and membrane filtration technique which lead to secondary waste streams which require further treatment. Both techniques are quite effective for color removal, but the cost and energy needed is rather extensive [4].

Microbial fuel cells (MFC) are a very potential technique as it does not only generate electricity but also have a great potential to accomplish wastewater treatment simultaneously. MFC have many operational and functional advantages over the technologies currently used to generate electricity. The usage of MFCs enable high conversion efficiency due to the direct conversion of substrate to electricity, as MFCs can operate at ambient and low temperature [6]. Besides, MFCs does not require gas treatment due to the off-gases of MFCs are enriched in carbon dioxide and lastly MFCs do not need energy input for aeration.

The purpose of this study was (i) to determine the efficiency of anaerobic bacterial strains isolated from Iraqi oil reservoir in degrading synthetic Azo dye AR27 in the absence of nitrogen source; (ii) to study the capability of anaerobic bacterial strain isolated from Iraqi oil reservoir in the production of electricity through MFC.

2. Material and method

2.1. Bacterial growth, inoculation and medium preparation

Bacterial strains (POS and PCO Oil) isolated from Iraqi oil reservoir were grown anaerobically for 24 h in a nutrient broth (NB) medium (Merck, Germany) with 8 g of Nutrient Broth per liter and 1000 ml of distilled water in an incubator shaker at 150 rpm and 37ºC. Synthetic wastewater medium: chemically defined medium (CDM) was used as inoculums for the bacterial POS and PCO Oil decolorization activity. K_2HPO_4 (7g/L), KH_2PO_4 (2g/L), $MgSO_4.7H_2O$ (0.1g/L), $CaCl_2$ (0.02g/L), glycerol (0.00, 1.27, 2.54, 3.804, 5.072, 6.34 g/L) and AR27 (0.1 g/L). Potassium ferricyanide, K_3FeCN_6 with molecular weight of 329.25 g/mol was prepared to obtain concentration of 0.1 mM. This K_3FeCN_6 solution was then diluted with 0.5 L of phosphate buffer (1 M, pH 7.0).

2.2. Decolorization of synthetic Azo dye AR27

Decolorization efficiency of synthetic Azo dye AR27 (50 mL) by bacterial strains POS and PCO Oil (0.8 absorbance at 600 nm) at different volume of bacterial strains consortium (1, 5 and 10 mL) was measured by monitoring the decrease in absorbance at the maximum wavelength of 521 nm using an UV-Vis spectrophotometer. Different glycerol concentrations, 0.00 g/L, 1.27 g/L, 2.54 g/L, 3.804 g/L, 5.072 g/L and 6.34 g/L, respectively, and different volume of bacterial strains consortium were

used in the CDM in order to monitor the decolorization efficiency. For the 240 h decolorization experiment, test sample were withdrawn at 0, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h and centrifuged at 10,000 rpm for 3 minutes prior to measurement of absorbance. Decolorization efficiency (%) = ((A-B)/A) x 100%, where A was initial absorbance, B was observed absorbance.

2.3. Setup and operation of MFC

A two chamber (anode and cathode) microbial fuel cell was used as a fuel cell system for the microbial electricity production. The total volume and working volume of each chamber were 250 mL and 200 mL, respectively. The electrodes, each of which was made up of 3 cm of the graphite rod was exposed to anode and cathode solution with radius 0.25 cm providing a working surface area of 5.105 cm^2. Copper wire, resistance and multimeter were connected in order to complete the circuit. The anode and cathode chambers were separated by salt bridge. In the fuel cell system, a 10% of bacterial cell suspension (0.8 absorbance reading at 600 nm) was used. The anode chamber was filled with CDM medium containing different concentration of glycerol (0.00, 1.27, 2.54, 3.804, 5.072, 6.34 g/L) and inoculated with bacterial strains consortium. Meanwhile, the cathode chamber contained phosphate buffer (1 M, pH 7.0) and 0.1 mM potassium ferricyanide, K_3FeCN_6 as electron acceptor.

2.4. Bioelectrochemical characterization

The voltage and current were measured using a multimeter (Victor VC 830 l). Measurements were performed periodically every 24 hours. Current density (mA/cm^2) was calculated from I = V/(RA), and power density (mW/cm^2) was calculated from P = VI/A, where R was resistance, V was cell voltage and A was the surface area of the anode. 2.54 g/L of glycerol concentration was used for the MFC operation after decolorization experiment has taken place.

2.5. Scanning electron microscope (SEM)

The graphite rod electrode that has been used in the running of MFC was analyzed using Hitachi Tabletop Microscope TM3000, Scanning Electron Microscope (SEM) for morphological analysis. The graphite rod electrode used in the MFC was removed at the end of the experiment and dried at high temperature, 70ºC. Electrode with 1 cm × 1 cm were cut and used for SEM analysis. SEM was carried out to observe the bacterial attachment to the anode surface.

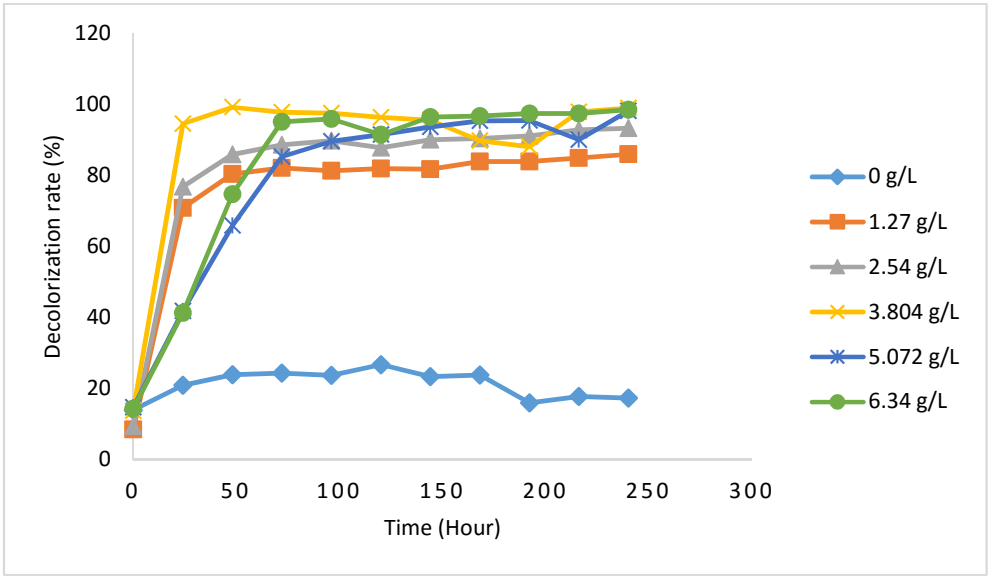
3. Results and discussions

3.1. Decolorization of synthetic Azo dye AR27

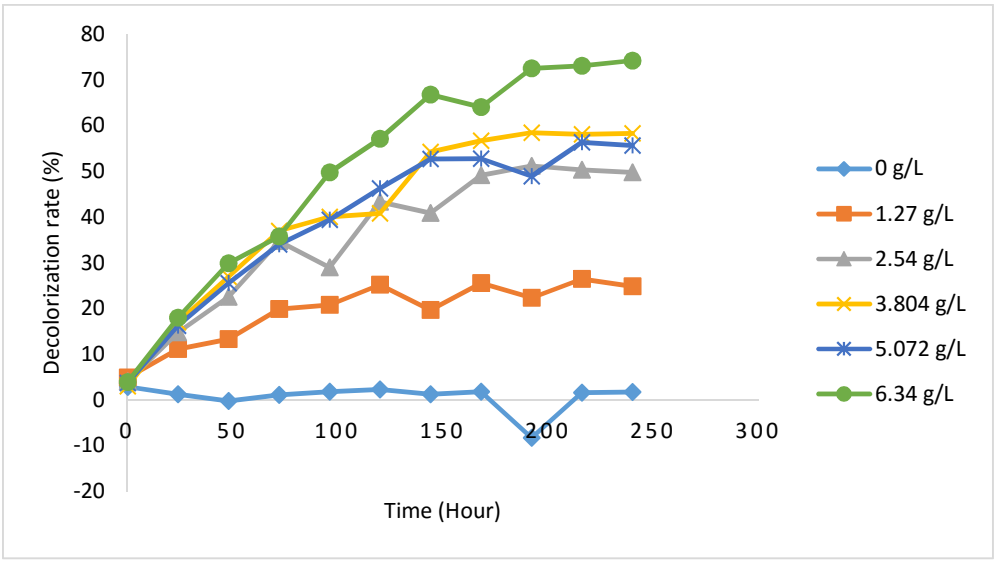
Decolorization of synthetic Azo dye AR27 were examined at different volumes of bacterial consortium POS and PCO Oil (1 mL, 5 mL and 10 mL) and glycerol concentrations (0.00 g/L, 1.27 g/L, 2.54 g/L, 3.804 g/L, 5.072 g/L and 6.34 g/L). Fig. 1 shows the decolorization rate of those two parameters.

As shown in Fig. 1 (a), 3.804 g/L glycerol concentration with 10 mL bacterial consortium solution (POS and PCO Oil) showed the highest decolorization rate at 99% for the first 48 hours. According to the bacterial population in Table 1, $3.9x10^8$ CFU/mL of colony were present in the original sample post decolorization. This implied that almost 100% decolorization can be achieved by the bacterial consortium (POS and PCO Oil). Decreasing the volume of bacterial consortium shows decrease in the decolorization rate.

As shown in Fig. 1 (b) and (c), at the first 48 hours, 27% decolorization was achieved from 5 mL and 1 mL bacterial consortium solution, respectively. After 120 hours, 10 mL, 5 mL and 1 mL bacterial consortium solution achieves 96%, 57% and 40% decolorization rate respectively at different glycerol concentration. This indicate that, a large amount of bacterial consortium solution is required to degrade the synthetic Azo dye AR27. Table 1 indicated the number of original sample post decolorization of synthetic Azo dye AR27. Nitrogen sources such as peptone or yeast extract could enhance the decolorization efficiency [7]. In contrast to nitrogen source, glucose inhibited decolorization activity because the consumed glucose was converted to organic acids that might decrease the pH of the culture medium, thus inhibiting the cell growth and decolorization activity.



(a)



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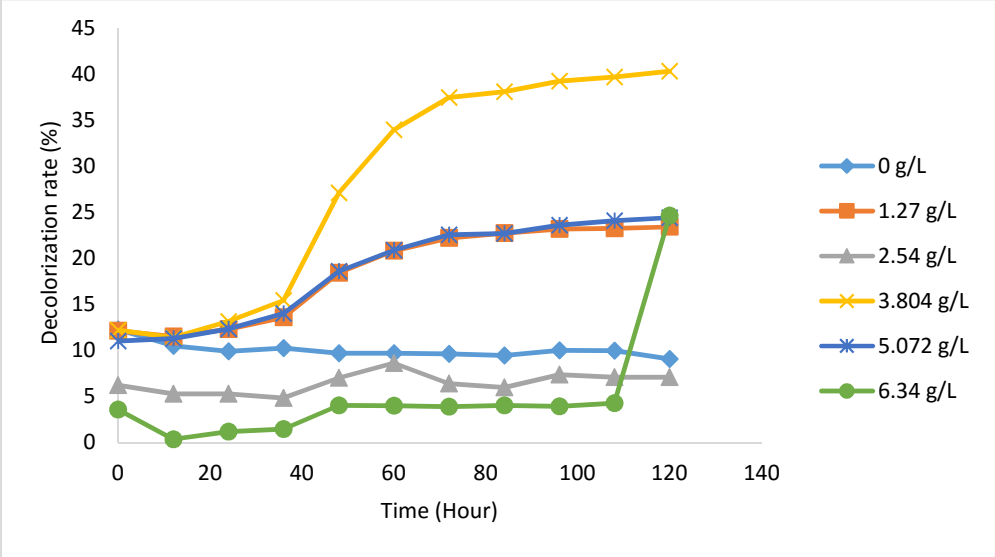


(c)

Fig. 1. (a) Decolorization rate of synthetic Azo dye AR27 using 10 mL bacterial consortium (POS and PCO Oil); (b) Decolorization rate of synthetic Azo dye AR27 using 5 mL bacterial consortium (POS and PCO Oil); (c) Decolorization rate of synthetic Azo dye AR27 using 1 mL bacterial consortium (POS and PCO Oil)

However, in this study, the absence of nitrogen source and the presence of high glycerol concentration did not affect the decolorization efficiency. Glycerol were used in this study instead of glucose as a substitute of carbon source. Similar to glucose, glycerol is also a simple sugar. According to [8] about 10% (w/w) glycerol is generated from animal fats and vegetable oils which excess glycerol may lead into an environmental problem since it cannot be disposed of in the environment.

Table 1
Percentage of decolorization rate with glycerol concentration

Volume of bacterial consortium (mL)	Glycerol concentration (g/L)	Percentage of decolorization (%)
10	3.804	99.1887
5	6.34	74.1887
1	3.804	40.3688

One of the possible application of glycerol is its usage as a carbon source and energy source for microbial growth in industrial microbiology [8]. The usage of glycerol has enabled the bacteria to grow anaerobically. Glycerol also have greater degree of reduction than sugars and offers the opportunity to obtain reduced chemicals like succinate and ethanol.
The decolorization occured at 37ºC to enhance the efficiency of bacterial consortium in degrading the synthetic Azo dye AR27. 20-45 ºC temperature range appeared to have produced a specific high decolorization rate with optimal temperature around 37 ºC. The conversion of dye decolorization remained constant at nearly 100% between 23-37 ºC, but dropped dramatically after the temperature was extended over 40 ºC [9].

3.2. Electricity generation

In this study, the optimized MFC was constructed using 150 mL volume anodic chamber and cathodic chamber separated by a salt bridge. The anodic chamber contains 135 mL CDM medium with synthetic Azo dye AR27 and 10% bacterial consortium POS and PCO Oil while the cathodic

chamber contains only 150 mL phosphate buffer with 0.1 mM $K_3Fe(CN)_6$ as the electron acceptor. The CDM used contained 2.54 g/L glycerol concentration which was selected based on its efficiency to decolorize synthetic Azo dye AR27.

It has been recognized that one of the important factors in generating high current density of MFC is the electron transfer from the bacteria to the anode. In this case, the addition of redox mediator or electron acceptor, which facilitates the process of transferring electron, is required for the efficient operation of a MFC. Potassium ferricyanide, K_3FeCN_6 has been extensively applied as effective mediator compound in many MFC studies. The redox couple of ferricyanide is well characterized and possesses a readily quantitative pigment in its reduced form. Therefore, potassium ferricyanide has been applied repeatedly as an electron mediator in a range of fuel cell and amperomatric systems [10]. The reduction of ferricyanide ion to ferrocyanide was catalyzed by accepting electrons from a membrane-bound dehydrogenase as represented in the Eq. 1.1 [11]. Ferrocyanide which is the reduced form of ferricyanide, is afterwards reoxidized at a suitable electrode resulting in current generation.

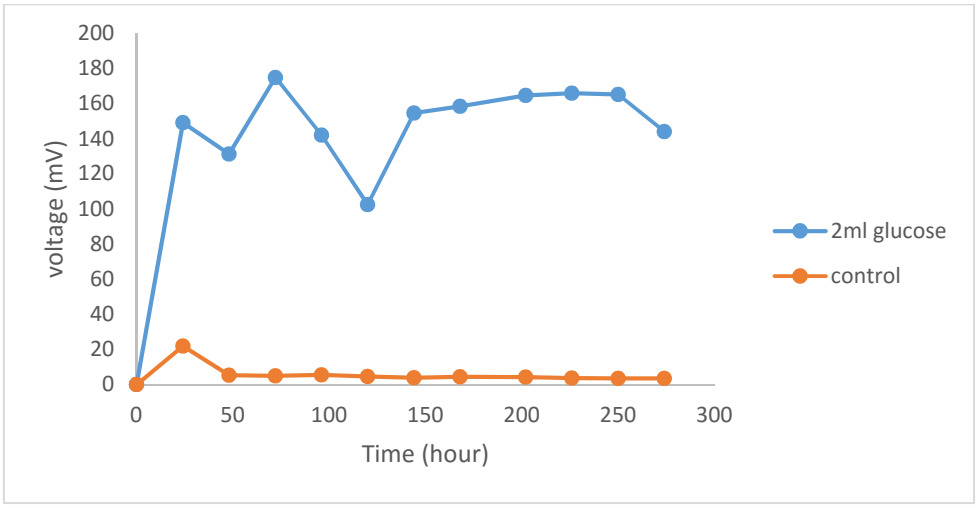
$$\underset{\text{(yellow)}}{Fe_{III}(Cn)_6^{3-}} + e^- \leftrightarrow \underset{\text{(colorless)}}{Fe_{II}(Cn)_6^{4-}} \quad 1.1$$

Based on Fig. 2 (a), the control MFC generate OCV with 21.9 mV within 24 hours while the studied MFC generated 175 mV OCV within 72 hours. This results proved the bacterial consortium POS and PCO Oil microbial process activity in the anodic chamber of studied MFC produced electrons and transferring them into the cathodic chamber simultaneously generating current. Based on the previous study, the cathode will also catalyze the cathodic reactions resulting in improved electricity generations [12].

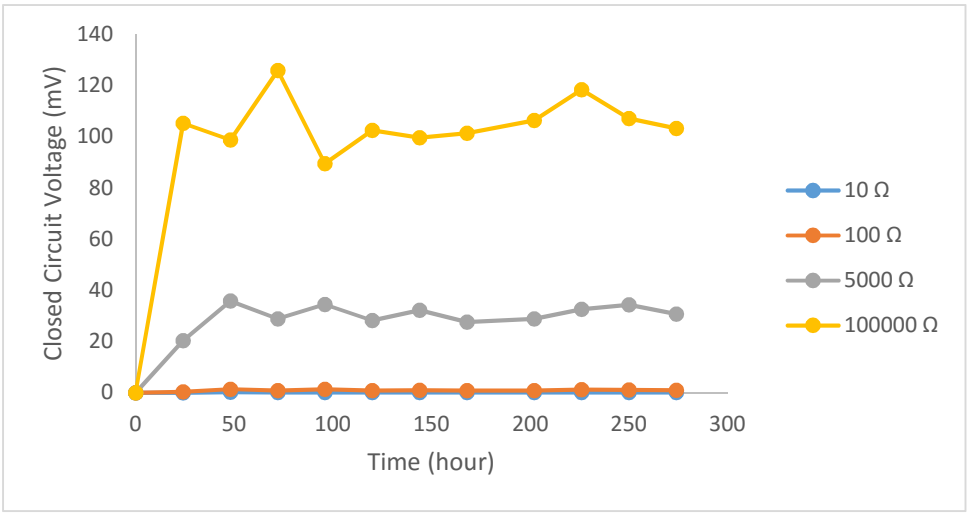
Fig. 2 (b) shows the voltage generation using different external resistor such as 10 Ω, 100 Ω, 5000 Ω and 100000 Ω. It was observed that with the change in the external resistor, the closed circuit voltage obtained was also affected. By increasing the value of external resistor, the voltage generation can be increased. 10 Ω external resistor produced maximum voltage of 0.2 mV while 100000 Ω external resistor produced 125.8 mV. Hence, from the data obtained, it was concluded that for the bacterial consortium POS and PCO Oil, 100000 Ω was observed as the optimum external resistor value and it was concluded that 100000 Ω would be equal to or close to the internal resistance of the constructed MFC. It has been reported that the external electric circuit must have a resistance equal to the internal resistance of the MFC to produce the maximum amount of power [13].

Table 2
Colony counting of bacterial consortium (POS and PCO Oil) after decolorization of synthetic Azo dye AR27.

Sample		10 mL bacteria + 3.804 g/L glycerol	10 mL bacteria + 6.34 g/L glycerol	10 mL bacteria + 2.54 g/L glycerol	5 mL bacteria + 3.804 g/L glycerol	5 mL bacteria + 6.34 g/L glycerol	5 mL bacteria + 2.54 g/L glycerol
No. of colony in original sample (CFU/mL)	Before	$2.6x10^8$	$3.9x10^8$	$2.07x10^8$	$5.3x10^7$	$9.8x10^7$	$3.04x10^7$
	After	$3.9x10^8$	$2.8x10^8$	$4.6x10^8$	$1.6x10^9$	$1.9x10^9$	$1.5x10^9$



(a)



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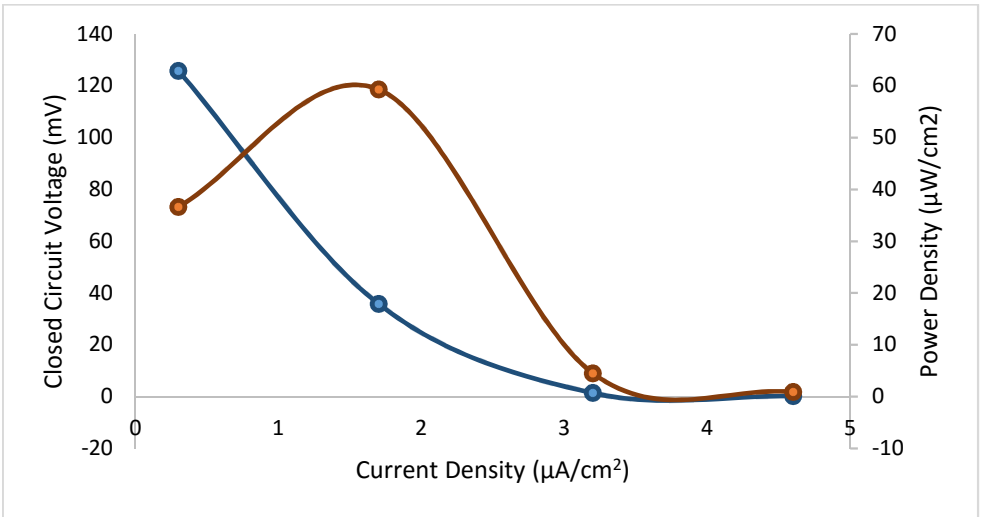

(c)

Fig. 2. (a) Open Circuit Voltage (OCV); (b) Close Circuit Voltage (CCV) generation at different resistor values; (c) Power density and current density curves.

As shown in Fig. 2. (c) and Table 2, the maximum power generated using ferricyanide was 256.3 µW at current value of 7.2 µA when an external load of 5 kΩ was applied. The same goes with the

maximum current density and maximum power density, 1.7 µA/cm^2 and 59.3 µW/cm^2 respectively, were achieved when an external load of 5 kΩ was applied. Previous study shows that maximum power increased by between 50% - 80% in the presence of ferricyanide [14]. Power output is much greater using ferricyanide as the electron acceptor in the cathodic chamber. So far, studies reported obtained very high power outputs such as 7200 mW/cm^2, 4310 mW/cm^2 and 3600 mW/cm^2 by applying the ferricyanide in the cathodic chamber [14-16].

3.3. Scanning electron microscope

The graphite rod of anode chamber was characterized by SEM to examine the bacterial attachment onto the graphite rod at the anode. Fig. 3 illustrates the morphologies of the anode surface. SEM images clearly show changes in anode surface prior and post MFC operation. SEM images shows smooth and clear anode surface (a) while confirming the biofilm formation on the anode surface (b).

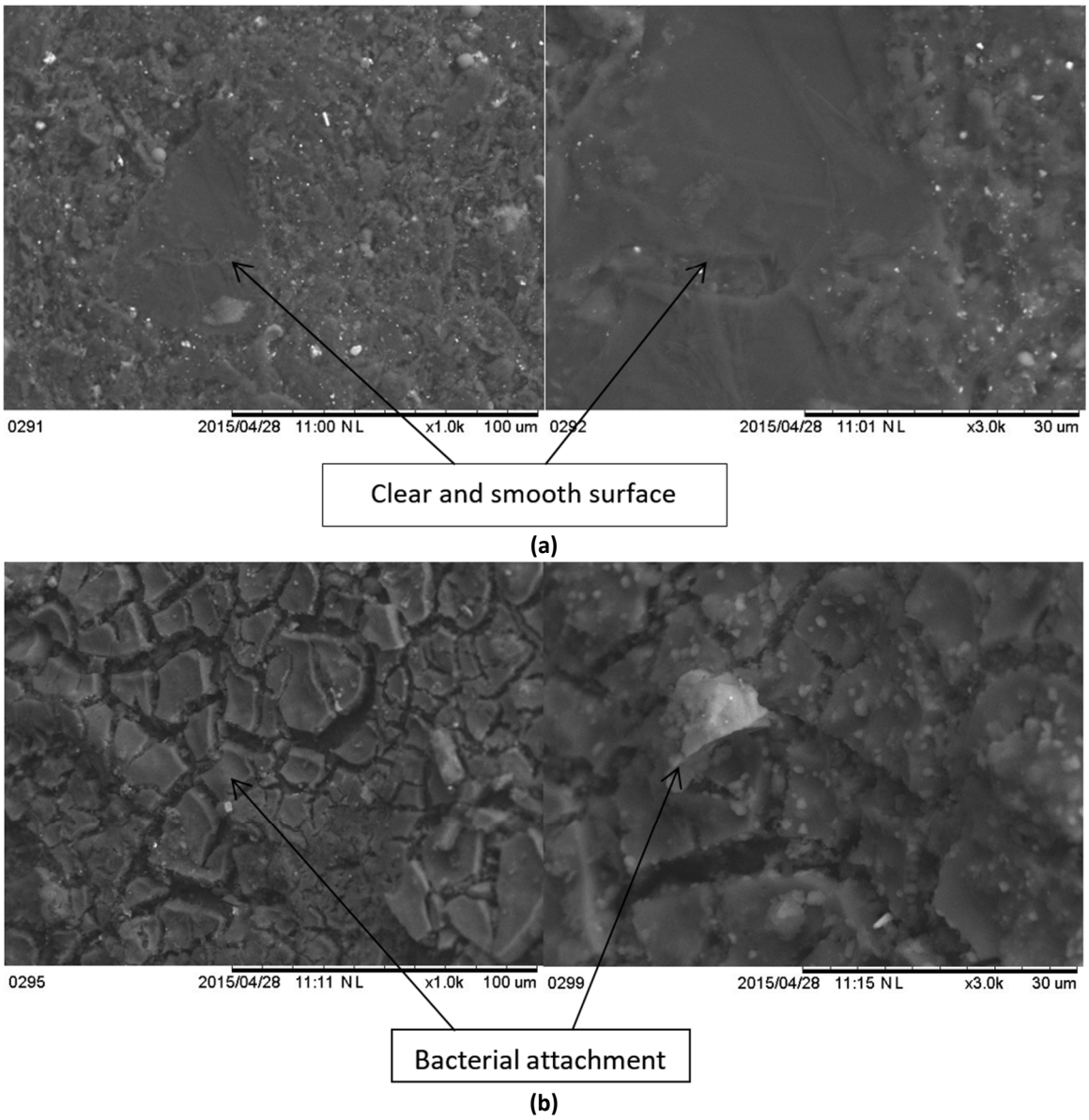

Fig. 3. SEM images of the anode surface before (a) and after (b) MFC operation.

Bacterial attached to the surfaces of electrodes were primarily accountable for current generation. The SEM observation shows that anodic electrode surface was covered by bacilliform bacteria which were responsible for electron transfer and thus current generation in the MFC [17].

Table 3
Current density and power density

Voltage (mV)	Resistance (Ω)	Current (I) (μA)	Power (μW)	Current Density (μA/cm^2)	Power Density (μW/cm^2)
0.2	10	200.0	4.0	4.6	0.9
1.4	100	14.0	19.6	3.2	4.5
35.8	5000	7.2	256.3	1.7	59.3
125.8	100000	1.3	158.3	0.3	36.6

4. Conclusion

Effective synthetic Azo dye AR-27 decolourization was realized in the absence of nitrogen source occurs primarily under anaerobic condition by bacterial consortium POS and PCO Oil. The most efficient decolorization rate of synthetic Azo dye AR27 was achieved with 10 mL bacterial consortium POS and PCO Oil in 50 mL conical flask volume containing CDM with 6.34 g/L glycerol concentration. Electricity generation is much greater using ferricyanide as the electron acceptor in the cathodic chamber with 256.3 μW, 7.2 μA, 1.7 μA/cm^2 and 59.3 μW/cm^2 when 5 kΩ external resistor was applied. This work showed that the bacterial strains POS and PCO Oil have an ability to decolorize Azo dye AR27 and generate electricity in the absence of nitrogen source.

LIST OF ABBREVIATION / SYMBOLS	
A	Ampere
e^-	Electrons
$Fe(CN)_6^{4-}$	Ferricyanide
g	Gram
g/L	Gram per liter
H	Hydrogen ion
K_2HPO_4	Dipotassium Hydrogen Phosphate
KH_2PO_4	Potassium Dihydrogen Phosphate
mV	MiliVolt
μV	MicroVolt
μA	MicroAmpere
μW	MicroWatt
^oC	Degree Celcius

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