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Effects of Yeast Concentration and Microalgal Species on Improving the Performance of Microalgal-Microbial Fuel Cells (MMFCs)

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Abstract – *This research aimed to determine the effects of various operating conditions such as yeast concentration (8–16 mg/L), anolyte pH (3–6) and type of microalga used (Spirulina or Chlorella) on the performance of microalgal-microbial fuel cells (MMFCs). MMFCs featuring a salt bridge, tofu wastewater in the anode chamber and microalga in the cathode chamber were constructed, and their performance was analyzed in terms of power density production, biomass growth, and chemical oxygen demand (COD) removal. Result showed that Spirulina produces a higher power density (maximum 0.98 mW/m*^{2}*) than Chlorella (maximum 0.39 mW/m*^{2}*). The COD removal rate in the microalgae coupled to microbial fuel cells operated at pH =3 was higher than those of MMFCs operated at other pH, and Chlorella showed higher carbon utilization from COD than Spriulina. A yeast concentration of 16 mg/L resulted in the best operating conditions.*

Keywords – *bioelectricity, microalgae-microbial fuel cell, power density, S. cerevisiae, tofu waste treatment.*

1. INTRODUCTION

Microbial fuel cells (MFCs) have received increased attention in recent years owing to their ability to produce electricity from soluble or dissolved organic wastewater [1]. Several wastewater sources have been investigated as bacterial substrates for MFCs and being considered as the future challenges and prospects of the energy recovery from wastewater [2].

In an MFC, microbes decompose the organic matter present in these wastewater and generate electrons. MFCs mainly decompose polluting organic matter in wastewater and generate electricity. Most MFCs consist of two compartments, *i.e.* cathode and anode compartments. In the anode chamber, active microorganisms such as bacteria are used as a biocatalyst to oxidize carbon sources and produce protons and electrons. Protons are transferred through proton exchange membranes (PEMs) or salt bridges, and the electrons are delivered through an external circuit to the cathode compartment [3]. Protons and electrons are reacted in the cathode compartment with the reduction of oxygen [4].

Microalgal biomass is a particularly interesting alternative to fossil fuels because of its high content of lipid and carbohydrate contents [5]. The utilization of microalgae as a source of biomass depends on the availability of nutrients and carbon sources; several studies have been conducted to investigate the use of wastewater as a nutrient and carbon source for microalgal growth [6]-[7]. The integration of wastewater treatment and energy production was previously demonstrated by Ichsan *et al.* [8], who elaborated on the use of palm oil mill effluents for biogas production.

The use of microalgae coupled to microbial fuel cells (MMFCs) has sparked intense research interest on account of the oxygen rich environment provided by these cells and the ability of microorganisms to utilize CO_2 in the cathode compartment as a carbon source in photosynthetic reactions [9]. Microalgae in cathode compartment could generate currents as result of oxygen production.

The main requirement of nutrients utilization in wastewater by *microalgae* is the C/N ratio in wastewater as well as in the algae biomass. Park *et al.* [10] showed that the C/N ratio in wastewater is approximately 3–7; in algae biomass, C/N ratio is 6–15; therefore, addition of an external carbon source is required. Integration of microalgae into MFCs by placing wastewater in the anode chamber and a microalgal culture in the cathode chamber, could supply carbon from bacterial activity in the wastewater to the microorganisms. In this way, the carbon limitation of alga-based treatment systems is addressed without the need for an external CO_2 supply. The reactions in an MMFC are as follows [11]:

$$\text{Anode}: C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24\,e^-$$

$$\text{Cathode}: 6CO_2 + 12\,H^+ + 12\,e^- \rightarrow -(C_6H_{10}O_5)-_n + 3O_2 + H_2O$$

Several studies have sought to increase the performance of MMFCs. Replacement of PEM with membrane-less systems such as salt bridges, glass fibers, porous fabrics, and coarse-pore filters has also been attempted to reduce operating costs [12]. For example, addition of yeast to the anode chamber resulted in remarkable increases in electron produced from wastewater decomposition [13]. Thus, the aim of the present study is to investigate the effect of various operating parameters, such as type of microalga used, anolyte pH, and yeast concentration on the power density and chemical oxygen demand (COD) reduction of an MMFC. In this work, the MMFCs employed included a salt bridge for proton transport.

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2. METHODOLOGY

2.1 Materials

Tofu wastewater was obtained from a local tofu producer in Semarang City, Central Java Indonesia. This wastewater consisted of soy protein residuals produced by the coagulation process.

Spirulina platensis and *Chlorella vulgaris* were cultured at the Center of Biomass and Renewable Energy (CBIORE), Diponegoro University, and nutrients such as $NaHCO_3$, urea, and TSP were used to help the microalgae grow. These microalgal strains were used for MMFC application at optical density (OD) of 0.6% ± 5% measured at 680 nm by a visible light spectrophotometer. Food-grade, Potassium chloride (KCl), mixed with agar, was used as a salt bridge to facilitate ion transfer between the anode and cathode compartments of the cell. *Saccharomyces cerevisiae* was provided by local bakery producers and used as yeast.

2.2 Preparation of the Salt Bridge

The salt bridge was prepared by using a PVC pipe (diameter = 1 inch; length = 8 cm). Exactly 2.33 g of agar powder and 150 mL of water were mixed in a 150 mL beaker glass and heated to 100°C until the solution reached the desired viscosity. KCl salt powder (2 M) was added to the agar mixture and stirred until complete dissolution was achieved. The mixture of agar powder and salt solution was poured into a beaker. The agar was allowed to harden, and the PVC pipe was inserted into the middle seal of MMFC reactor.

2.3 Configuration of MMFC

Two identical MFCs used were constructed from two glass bottles of equal volume (0.25 L). A glass channel (diameter, 2 cm; length, 8 cm) in between a salt bridge connection fixed in the middle (Figure 1). A 24.5 cm long carbon brush (diameter, 5 cm) with a 19 cm long Ti handle was used as the anode electrode, and the cathode electrode was constructed from a piece of carbon fiber cloth (64 x 55 x 2 mm^3; surface area 75.16 cm^2). The anode and cathode electrodes were connected by wires of Ti (inside the bottle) and Cu (outside the bottle) with an external resistance of 1000 W.

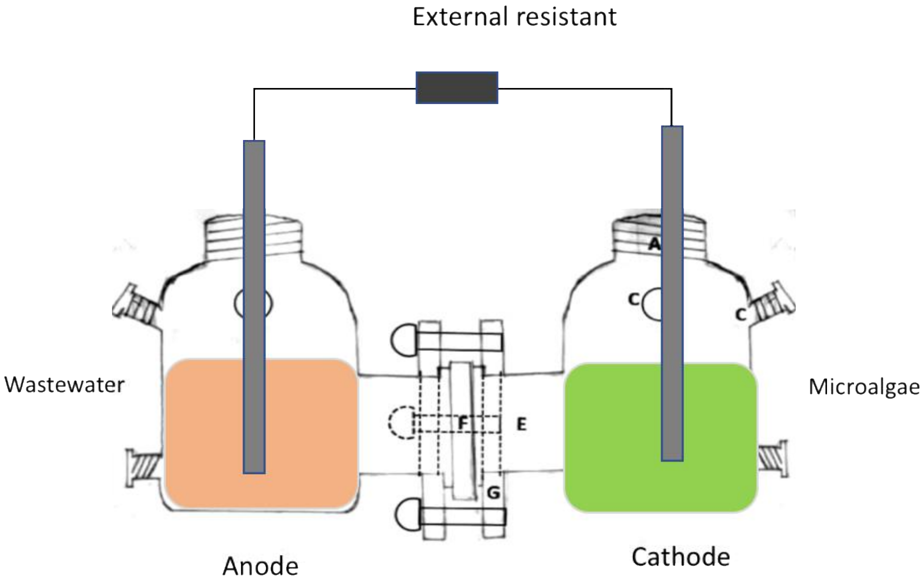

Fig. 1. Experimental set up of microalgae microbial fuel cell (MMFC).

2.4 Microalgal-Microbial Fuel Cell Operation

The anode was filled with 200 mL of tofu wastewater mix with an initial COD of approximately 2000 mg/L. The cathode chamber was filled with 250 mL of *Spirulina* or *Chlorella* during the first 12 days of MFC operation. *S. cerevisiae* was added to tofu wastewater at a concentration of 8, 12 or 16 mg/L.

The MMFCs were operated at 30°C, under continuous light (5,000 lux) for 14 days per batch until the growth of microalgae ceased. DO and pH values were measured, and pH adjustment to 7 was conducted using 1 M HCl or NaOH every 2 days, as necessary. The OD of the microalgae and the current, COD and voltage of the cells were measured every day.

2.5 Analytical Method

The working voltage and current of the MFCs were monitored by using a voltmeter and ammeter connected to the cells. Current density and power density at external resistance of were normalized with the anolyte volume. Polarization curves were measured by recording the voltages generated under various external resistors from 10 Ω - 47,000 Ω using the procedure applied in a previous analysis of polarization tests conducted for fuel cells [14]. Power density was calculated using the formula:

$$P = V \times I / A \tag{1}$$

where P represents the power density, V is the voltage, I is the electric current, and A is the surface area of the anode.

OD was measured by a spectrophotometer (HI 83224; Hanna Instruments) at 530 nm. The COD of the wastewater was measured by a thermodigester (HI 839800; Hanna Instruments).

3. RESULTS AND DISCUSSION

3.1. Effect of Microalgal Species on Power Density

Figure 2 shows the power densities produced by *C. vulgaris* and *S. platensis*.

During the first 5 days of operation of the MMFCs, the power density of both microalgae increased. The maximum power density of *Spirulina i.e.* 17.6 mW/m^2 is higher than that of *Chlorella i.e.* 7.4 mW/m^2. Differences in the power densities of these species are caused by differences in their capability to absorb light and grow. *Spirulina* has higher light absorption capacity than *Chlorella* [15]. Moreover, as reported by Margarites *et al.* [16], the growth rate and productivity of *Spirulina* are higher than those of *Chlorella*. The greater the biomass productivity, the greater the energy conversion from the algal biomass and the conversion of chemical energy into electrical energy. Electricity could be produced by cathodic algae growing in MFCs, where oxygen is produced by photosynthesis [17]. Algal productivity at the cathode could lead to greater acceptance of protons from the anode [18]. The lack of electrons in the cathode could attract electrons from the anode and consequently, increase the power density of the cell.

3.2 Chemical Oxygen Demand (COD) Removal

Figure 3 shows a decrease in COD after MMFC operation. *Chlorella* shows better ability than *Spirulina* to utilize CO$_2$ from wastewater decomposition (COD removal rate, 47.8% vs 32.1%). Microalgae require a carbon source to maintain their metabolism. In the present case, the carbon source was provided by COD removal in the anode chamber.

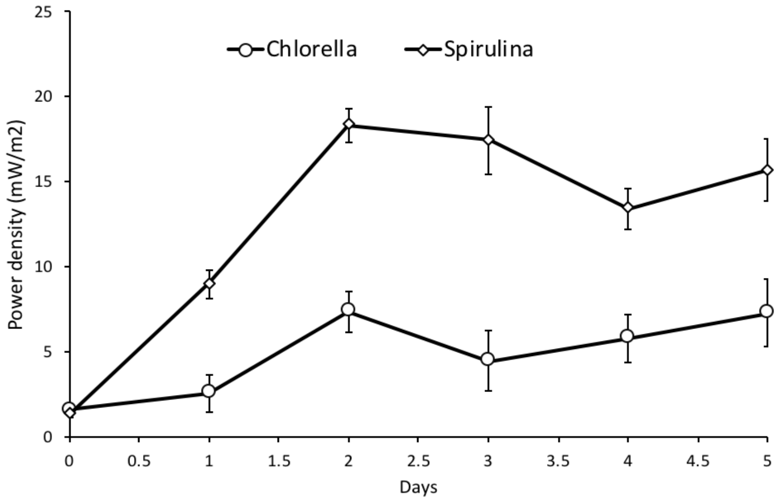

Fig. 2. Comparison of voltage production in MMFCs with *Chlorella vulgaris* and *Spirulina platensis* over time.

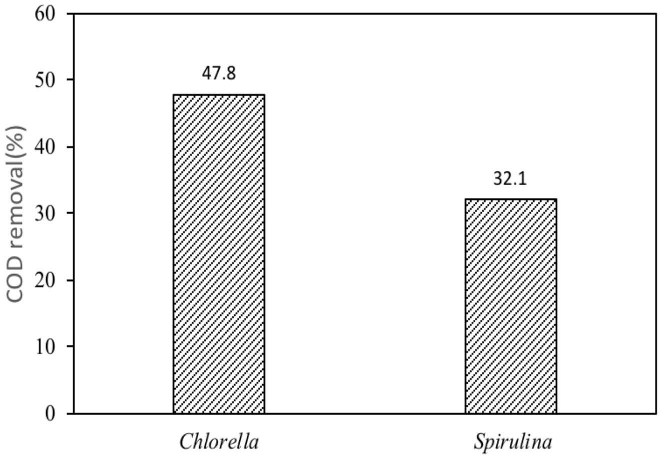

Fig. 3. COD removal rates achieved in MMFCs with *Chlorella vulgaris* and *Spirulina platensis.*

COD removal from wastewater could also be attributed to the adsorption of organics by a large number of microspores on the surface of the electrode [19]. The removal of COD confirms that the contents of wastewater, especially organic compounds, could be degraded by bacteria and be utilized as a nutrients in the growth of *Chlorella* and *Spirulin*. In the authors' experiment, there was no significant change in biological oxygen demand (BOD) removal was observed when dose of *C. vulgaris* was increased from 1 g/L to 10 g/L. In general, the microalgal concentration is not known to remarkably increase COD removal rates [20].

3.3 Effect of Anolyte pH on Microalgal-Microbial Fuel Cell Performance

An MMFC is a device designed to produce electricity by integrating waste degradation and algal cultivation. Besides, the type of microalga used, pH also plays important role in the MMFC performance.

Figure 4, shows that the voltage produced by an MMFC operated at pH 4 is lower than those produced at pH 3 and pH 6. The cell voltage in a galvanic fuel cell is determined by the difference between the oxidant (cathode) voltage and the fuel (anode) voltage. The production of electrons in microbial fuel cell is made possible by the oxidation of NADH to NAD$^+$ and the release of electrons from NADH to the electric circuit [21]. It is observed that the potential to reduce NAD$^+$ to NADH is equal to -0.32 V in pH 7 and 0.094 V in pH 1 [22]. For MMFC operations in which NADH is oxidized to NAD+, the potential is positive at pH 7 (0.32V) and negative at pH 1 (-0.094 V). Therefore, acidic conditions favor the MMFC performance of the MMFC. Assuming a constant oxidant/cathode voltage, the cell voltage increases at low pH.

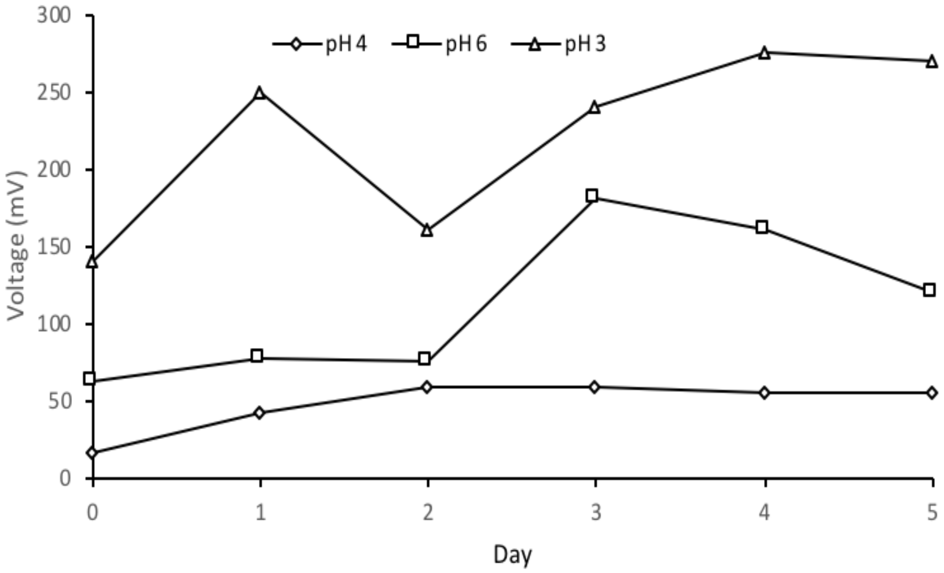

Fig. 4. The effect of anolyte pH to voltage resulted in MMFC.

Current flow is closely related to solution conductivity. Increasing the ionic strength in an MMFC promotes electricity production [23]. This statement is representing the fact that pH 6 anode chamber has greater current compared to pH 4. The addition of NaHCO3 endows the fuel cell with a stronger ionic strength, which could improve its conductivity and current flow. At low pH, the ionic strength of the MMFC is provided by H$^+$ instead of sodium ions. While sodium only increases conductivity of the FC, H$^+$ acts as both a reactant and conductivity booster. Lower pH could contribute to the availability of H$^+$ that may be reduced, and hence increase current production.

Because power is proportional to both current and voltage, the power density of an MMFC is also proportional to its voltage and current production. Among the different pH values studied, the anode solution of pH 3 yielded the highest power production, despite its fluctuation. The anode solution of pH 6 yielded high power after the second day of operation. The power density produced in the anode solution of pH 4 (5 mW/m^2) was much lower compared with those produced at other pH. The figure in this research is comparable to the power density reviews, cells producing around 35-45 mW/m^2 [24], similar cell configuration producing around 60-80 mW/m^2 [25]. Power densities ranging from 20 mW/m^2 to 1.9 W/m^2 may be obtained using the most advanced electrodes [1]. Most MFCs are targeted to remove organic or heavy metal waste inside their anode solution. pH is an important factor in the decomposition process of COD. According to [26], the optimum pH for COD removal by is at pH 4-5. This finding is quite different from the results of the present study. In the authors' experiment, the highest COD removal was achieved at pH = 3 (Figure 4). At normal condition of pH 4, COD removal reaches 25%; however at pH 6, it is only 11% of the COD available that was successfully removed. At pH 3, the COD removal rate reached 37%. According to Pena *et al.* [27], the yeast of *S. cerevisiae* grows rapidly in acidic conditions. Without buffers, yeast cultivated under optimum conditions may not grow longer when the medium becomes excessively acidic. Thus, a medium that is slightly more alkaline than its optimum pH of the yeast may be more beneficial to the latter's growth. At pH 3, the yeast shows an exponential growth rate [28]. Acetic acid is added as an acidity regulator to solutions of pH 3 to form a buffer that could help stabilize the cultivation medium and support COD removal.

3.4 Effect of Yeast Concentration on MMFC Performance

The effect of yeast concentrations ranging from 8 mg/mL and 16 mg/mL on the performance of the MMFC was evaluated. The maximum power density generated by the cell was observed on day 1 under a yeast concentration of 12 mg/mL (41.35 mW/m^2), on day 4 under a yeast concentration of 16 mg/mL (70.12 mW/m^2) and on day 5 under a yeast concentration of 8 mg/mL (15.90 mW/m^2).

Microalgae could be used in MMFCs to generate electric currents [29]. An MMFC employing *Spirulina sp*. at the cathode as an electron acceptor and S. *cerevisiae* as an electron donor was constructed, and its performance was tested. Large amounts of CO_2 produced by the fermentation process of yeast and native anaerobic microbes were transferred to the cathode side of the cell for microalgal growth and resulted in high ODs. The greater the yeast concentration used in the experiment, the greater the transfer of donor electrons at the anode are transferred to the cathode as an electron acceptor. The greater the number of electrons received, the higher the voltage, electric current, and power density produced. The voltage, electric current, and power density produced by the MMFC with a yeast concentration of 16 mg/mL are greater than those produced at other yeast concentrations. This finding confirms that higher yeast concentrations result in more electrons produced and, in turn, higher electric currents. So, even with the smallest yeast concentration, 8 mg / mL, based on experimental results, those variable has a smaller value of voltage, electric current, and power density due to the small transfer of electrons from the anode to the cathode.

Microorganisms require carbon sources to maintain their metabolism, and thus promote high COD removal. When the COD concentration is decreased, yeast must consume biochemical matter in the wastewater such as carbon, nitrogen, and phosphate to produce protons and electrons.

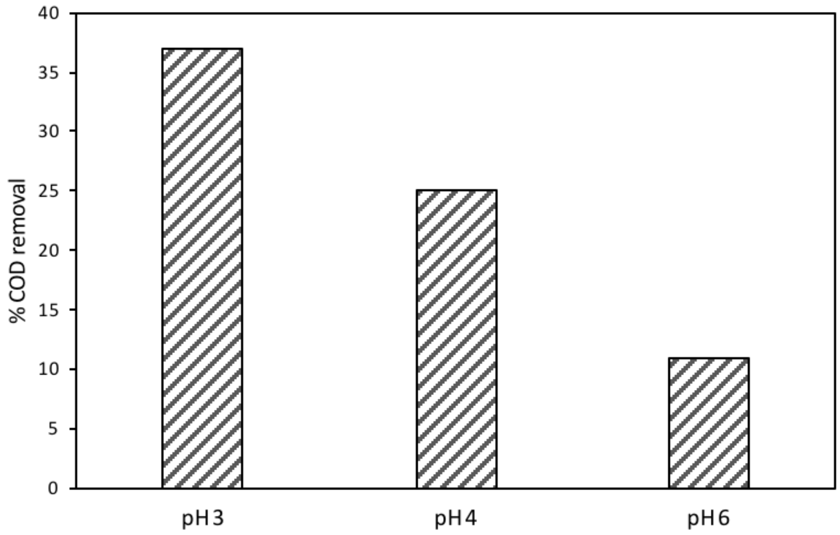

Fig. 5. % COD removal of different pH of anode in MMFC.

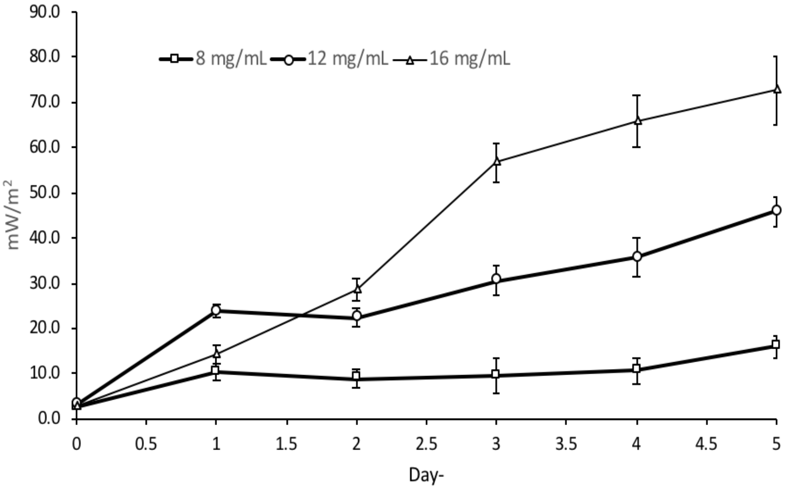

Fig. 6. Power density of MMFC with various anode yeast concentration

3.5 *Polarization curve*

The polarization curve shows the negative relationships between current and voltage. As the external resistance increases, the value of voltage increases whereas the current decreases [14].

The declining data may be explained by a number of reasons [14]:

- At low currents, the voltage drops rapidly as the energy of the cell is used to activate reactions
- Ohmic resistance losses account for linear and smooth decreases in voltage and increases in current.

The polarization test can help determine the characteristics of a fuel cell can be found to obtain maximum power density and coulombic efficiency. Figure 7 shows the phenomenon of a power overshoot. In a power overshoot, the potential and current density drop rapidly because microbes at the anode side of the cell cannot produce sufficient currents at a low potential [41], [42]. Substrate depletion and increased internal resistance also play important roles in the overshoot phenomenon.

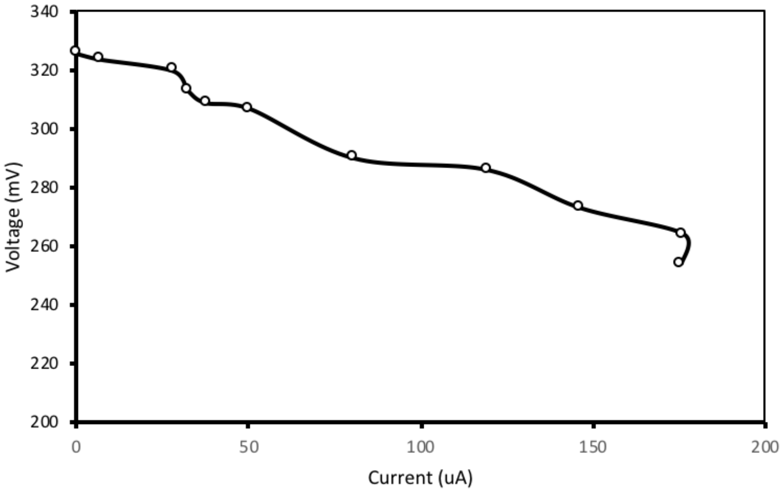

Fig. 7. Polarization curve of MMFC operation

4. CONCLUSION

The experimental results indicated that the voltage, current, and power density of the microalgal species, anode chamber pH, and yeast concentration variable tend to increase during the day. The maximum voltage, current, and power density were produced by using *Spirulina* sp., an anode chamber pH of 3, and yeast concentration of 12 mg/mL. The highest % COD removal rate from microalgal species was achieved by using *Spirulina* sp., an anode chamber pH of 3, and yeast concentration of 8 mg/mL.

Some suggestions for researchers conducting future experiments are as follows. First, analyze the nutrients in wastewater prior to starting the MMFC experiments. Second, use a sensitive multimeter to measure the voltage and current of the MMFC. Third, different acid solution with same pH value is used for the other kind of variable. Fourth, use an accurate pH meter to determine the pH of the wastewater at the beginning of the experiments. Finally, add all necessary nutrients to the microalgal solution once every 3 days during the experiment.

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