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Red Algae (*Eucheuma cottonii*) Extract as a Substrate in Microbial Fuel Cell Technology to Generate Electricity

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Abstract. The continuous use of oil and gas fuels causes a global energy crisis and environmental pollution, so it is necessary to use renewable and environmentally friendly energy sources. Microbial fuel cell (MFC) is a technology that can generate electricity by converting chemical energy into electrical energy using microorganisms as a catalyst. This research aimed to determine the potential of red algae (*Eucheuma cottonii*) as a substrate in MFC to generate electrical energy. This research used a dual-chamber MFC model. Anode chamber contains hydrolyzed red algae cellulose, peptone, yeast extract, KH_2PO_4, and methylene blue (this mixture is autoclaved before being put into the anode chamber), while the cathode chamber contains 0.2 M $KMnO_4$ as the electrolyte solution. This reactor used Nafion 117 membrane as a proton exchange membrane. The electrical measurement was carried out every 4 hours for 48 hours. The highest power density using red algae as a substrate was 982 mW/cm^2 with a value of maximum current 0.52 mA and potential different 370 mV. It is concluded that red algae (*E. cottonii*) extract can be employed as a subtract in the MFC system to produce electrical energy.

INTRODUCTION

The occurrence of a global energy crisis and environmental pollution due to the continuous use of oil and gas fuels so that there is a need for renewable energy sources that can provide a solution to this problem [1]. In recent years MFC has received much attention due to their ability to decompose organic matter and generate electricity [2] simultaneously. This bioreactor can convert chemical energy into electrical energy using microorganisms as catalysts in anaerobic conditions [3].

The working principle of MFC is that the electrons in it come from the oxidation of organic compounds by microbes [4]. This bioreactor consists of anode, cathode, and proton exchange membrane [3]. The resulting electrons are transferred to the cathode through an external circuit while protons are transferred internally through a proton exchange membrane [5]. Several factors can influence the performance of this bioreactor, such as type of substrate, membrane [6], microorganism [7], electrolyte solution [3], and mediator [8].

The energy produced can be obtained from various types of substrates such as cellulose [9], glucose [10], soil, marine sediment [6], wastewater [2,5,11], and whey substrate [12]. The commonly used membranes commonly used is proton exchange membrane (PEM) Nafion because of its mechanical stability and high proton and thermal conductivity of the membrane [6,11]. However, some other typical membranes have been used before, like salt bridge [1] and sulphonated poly (ether ether ketone) or SPEEK [13]. As mentioned previously, the use of microorganisms

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can oxidize organic matter and transfer an electron to the electrode. Several types of microorganisms have been used before, such as yeast (*Saccharomyces cerevisiae*) [10], *Lactobacillus bulgaricus* [12], and *Pseudomonas aeruginosa* [7]. The addition of electrolyte solution can affect the results of the energy produced [1].

Many studies have added electrolyte solutions in the MFC system to increase the efficiency of the resulting energy. Like Muftiana et al. (2018) used tofu wastewater as a substrate with *L. bulgaricus* [12]. The present research aims to determine the effect of increasing the concentration of $KMnO_4$ and $K_3[Fe(CN)_6]$ electrolyte solution. The experimental results showed that 0.2 M KMnO4 solution produced a higher potential difference of 99.2 mV, compared to 0.2 M $K_3[Fe(CN)_6]$ electrolyte solution with a potential difference of 48.6 mV. Some previous research used a mediator to improve the performance of MFC, like Yuan et al. (2020) with research "Co-Generation System of Bioethanol and Electricity with Microbial Fuel Cell Technology" glucose substrate with yeast (*S. cerevisiae*), whose results showed that the maximum power density value with the addition of MB (methylene blue) was 5.2 ± 0.5 W/m^2 [10]. This is supported by the result of research that has been done by Permana et al. (2015) which compares the using and not using methylene blue; the results of the research show that MFC with methylene blue produces a current of 5.5×10^{-5} A, a potential difference of 0.886 V, a power density of 4.48×10^{-3} W/m^2 while those that not using methylene blue produced a current of 5×10^{-5} A, a potential of 0.689 V, a power density of 2.12×10^{-3} W/m^2 [8].

This paper will use red algae *E. cottonii* as a substrate in MFC. This type of red algae is widely cultivated in South Sulawesi [14]. Based on the research results of Dompeipen and Dewa (2015), the cellulose content of the *E. cottonii* sample is 11.11%, so that it has the potential as a source of glucose in fermentation in the MFC system [15]. Based on the previous explanation, an investigation to reveal red algae (*E. cottonii*) extract potency in generating electrical energy in the MFC system and determine the optical density of yeast (*S. cerevisiae*) seems to be necessary.

MATERIALS AND METHOD

Materials and Tools

The material used in this research was the red algae *E. cottonii* obtained from fishers in Takalar, South Sulawesi, as a substrate in the MFC system. The microorganism used was yeast *S. cerevisiae.* Aquadest (H_2O), Nafion 117 (DuPont Co) as proton exchange membrane, hydrogen peroxide (H_2O_2), graphite electrode, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4), peptone, yeast extract, methylene blue, glucose, potassium permanganate ($KMnO_4$) and filter paper. The tools that will be used in this research include Spectrophotometer Visible (Thermo Scientific Genesys 20), oven (Memmert), autoclave, analytical balance (Kern), shaker incubator (Thermo Scientifc MaxQ4000), digital multimeter (Aneng A830L), grinding machine, electric heater, double chamber MFC bioreactor, glassware, cable, crocodile claw, spatula, and tube rack.

Methods

Manufacture of MFC Bioreactor

An MFC reactor was prepared, consisting of two chambers, namely the anode chamber and cathode chamber separated by a Proton Exchange Membrane (PEM) Nafion 117. Each chamber can hold 1 L and having holes with the diameter of each chamber are 3.5 cm. PEM was installed in the hole as a proton exchange site. Then 5 graphite electrodes were installed in each chamber and connected by a series of wires on a digital multimeter. MFC bioreactor made from acrylic with size 10 x 10 x 10 cm [14].

Preparation of Proton Exchange Membrane and Preparation of Graphite Electrode

The proton exchange membrane used is Nafion 117 (DuPont). The membrane was pretreated before being used on the MFC by boiling it using aquadest (H_2O) for an hour and then boiled with 3% H_2O_2 for an hour and rinsed with aquadest. Then the membrane was boiled again with 1 M H_2SO4 for an hour and rinsed with aquadest three times. Membrane stored (soaked) with aquadest until ready for use. Before being used in the MFC reactor, the membrane is dried by aerating [14]. The graphite electrode rod was immersed in 1M HCl for 1 x 24 hours and rinsed with aquadest. After that, the graphite electrode was immersed again with 1 M NaOH for 1 x 24 hours. Then it was rinsed with aquadest until it is ready to use [16].

MFC Experiment

Red algae were washed thoroughly with running water and cut into small pieces, and dried. Then the red algae were mashed using a grinding machine until it became a fine powder. Then it was sieved using a sieve shaker with a size of 100 mesh [16]. The process of hydrolysis of cellulose into glucose was carried out by adding 25 g of *E. cottonii* cellulose to 500 mL of 7% H_2SO_4. The mixture was heated at 100˚C for an hour while stirring, then filtered [17].

40 mL of the result red algae glucose hydrolysis, 5 g of peptone, 5 g of yeast extract, 5 g of KH_2PO_4, and 2 mL of methylene blue (MB) in 800 mL and put into anode chamber. The pH value of the anolyte was initially adjusted to 7.0 with 5 M NaOH. Its solution was sterilized at 121 °C for 15 minutes then cooled before use. A total of 1 g of yeast was cultured for an hour at 37 °C in 20 g/L of glucose solution. After that, the yeast was added into the anolyte then transferred to the anode chamber. The catholyte contains 800 mL of 0.2 M $KMnO_4$ and transfers to the cathode chamber. After that, the chamber cover was installed and connected to the voltmeter using crocodile clamps [10].

Data Analysis

The measurement of Yeast growth utilizes the optical density method using a Spectrophotometer Visible with a wavelength of 600 nm for 48 hours with time intervals every 4 hours [10]. Measurement of the potential difference and current generated every 4 hours for 48 hours using a digital multimeter. The current and potential different data can obtain the power value per unit electrode surface area.

$$\text{Value of Power Density} = \frac{V \times I}{A} \quad (1)$$

RESULTS AND DISCUSSION

The result of this research was substrate of red algae (*E. cottonii*) extract on a Microbial Fuel Cell which could produce current and potential differences. The type of MFC used in this study was dual-chamber MFC, which is consists of two chambers, namely the anode and cathode, where the anode contained red algae extract substrate. In contrast, the cathode contained an electrolyte solution ($KMnO_4$).

The Value of Optical Density (OD) Yeast in Media

The pattern of bacterial growth is needed because it determines the process of degradation of organic matter. The growth pattern of bacteria consists of four phases: the lag or adaptation phase, the log or exponential phase, the stationary phase, and the death phase [18]. In this study, media such as yeast extract, peptone, KH_2PO_4 were used as a medium growth of yeast (*S. cerevisiae*) and then shaken for 48 hours, where every 4 hours sampling and absorbance was measured using a Spectrophotometer Visible with wavelength 600 nm. The measurement results are in Fig. 1.

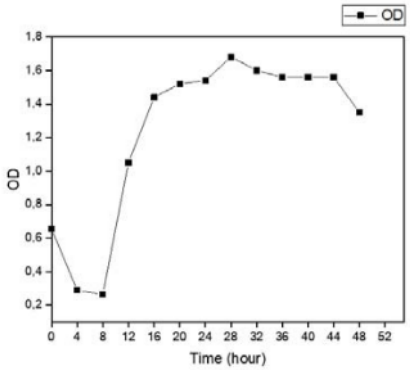

FIGURE 1. Yeast growth curve of *S. cerevisiae* in media

Fig. 1 shows the growth of *S. cerevisiae* during 48 hours of incubation. From the figure at 0 to 8th hour with OD value 0.637, 0.289, and 0.265, respectively, the OD value decreased; this indicates that the growth at the beginning of the incubation time was still not stable. This unstable growth was the influence of bacteria in the lag or adaptation phase. In this phase, the yeast will adjust to the new environment. Several factors influencing the lag phase comprise the inoculum's size, the cell's physiological history, and the exact physicochemical environment [19]. Based on the result of research from Yuan et al. (2020), there was a decrease in the value of OD because the rate of cell adsorption to the electrode was faster than the rate of growth [10]. From the 12th hour to the 28th hour, OD values were 1.050, 1.440, 1.520, 1.540, and 1.680. There was an increase in the OD value because the bacteria had entered the exponential phase. According to Burns (2016), the exponential phase is the increase in the amount of cell biomass very rapidly [20], on the curve above the 12th hour until the 28th hour, an increase in the OD value. From the 32nd hour to the 48th hour with OD value 1.600, 1.560, 1.560, 1.560, and 1.350, it had entered the death phase; namely, the death rate exceeds growth rate resulting in a net loss of viable cells as shown on the curve, the OD value is decreasing [20].

Measurement Result of Potential Different and Current

At this stage, the conducted experiment was on the media by giving yeast (*S. cerevisiae*) on red algae (*E. cottonii*) extract substrate and MB as a mediator. Using MB as a mediator was due to its ability to significantly increase electron transfer, extract overall energy, and modulate cell catabolism in the fermentation process [10]. Measurements of potential (mV) and current (mA) were carried out every four to 48 hours. The potential different (mV) measurement results are presented in Fig. 2, and the current (mA) measurement results are presented in Fig 3.

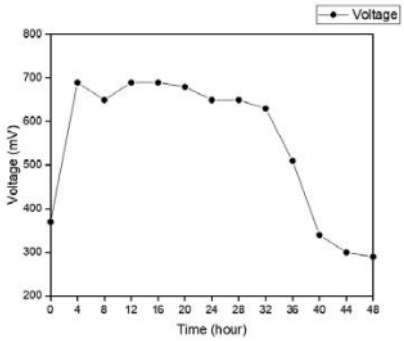

FIGURE 2. Potential differences in red algae (*E. cottonii*) extract substrate with electrolyte solution $KMnO_4$.

Fig 2 shows the value of the potential difference generated by the MFC system using red algae extract as a substrate; the red algae extract substrate using yeast *S. cerevsiae* as a biocatalyst gave a maximum potential difference (mV) from at 12th hour to 16th hour is 690 mV. At 0 to 8th hour, it showed a pattern of increasing and decreasing potential differences so that the resulting potential difference was not stable. The substrate used was glucose, which in the previous step, cellulose produced from the red algae *E. cottonii* did not go through the delignification process. So, there were still lignin and hemicellulose in the previous step, the substrate which inhibited the breakdown of glucose by *S. cerevisiae* into ethanol [18]. At the 12th and 16th hours, there was an increase in the value of the potential difference (690 mV). The maximum potential different value generated in this research was higher than the research results by Yuan et al. (2020) using glucose substrate, which was 457mV [10]. According to Jiang and Li (2009), substrate concentration may increase the output power generated [21].

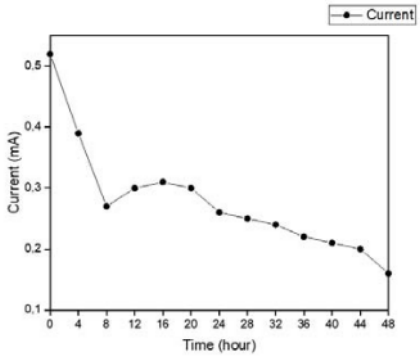

FIGURE 3. Current in red algae (*E. cottonii*) extract substrate with electrolyte solution $KMnO_4$.

Fig 3 shows the current value generated by the MFC system using red algae extract as a substrate with yeast *S. cerevisiae* which was measured every 4 hours for 48 hours. Measurements at 0 to 8th hour decreased because the bacteria in the lag phase were still adapting to the environment [22]. At the 12th hour to 16th, the current value increased because the bacteria had entered the exponential phase where there was a very rapid increase in cell biomass [23]. According to Arbianti et al. (2013), the value of current increases because the cell is actively metabolizing and the glucose level is still high, which allows a high rate of electron transfer [24]. At the 20th hour to the 48th hour, there was a constant decrease in the value of this current. It indicates that the bacteria had entered the death phase [12]. The maximum current value obtained from the red algae extract substrate was 0.52 mA. The current value obtained in this research is higher than the current value generated by Permana et al. (2015), which used glucose as a substrate, 5.5 x 10^{-5} A [8]. The current value obtained is related to several factors that could improve MFC performance, such as substrate concentration, electrode area, MFC design, membrane type, and others [2,6,21].

In this study, the substrate used was glucose from the hydrolysis of red algae cellulose. Yeast *S. cerevisiae* as a biocatalyst in MFC, uses glucose as a carbon source in the anode chamber and produces electrons (e^-) and protons (H^+). 24 moles of electrons and hydrogen ions were produced from the oxidation of 1 mole of glucose under anaerobic conditions. The reaction that took place at the anode is in the following equation [8].

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+ \quad (2)$$

Electrons move to the anode surface, and protons pass through the PEM membrane and react with oxygen at the cathode. Electrons from the anode were captured by permanganate ions (MnO_4^-) which act as electron acceptors. A permanganate ion is used as an electron acceptor because it has a high oxidation capacity and is environmentally friendly. Under either acidic or basic conditions, the permanganate ion accepts three electrons and is reduced to manganese dioxide, which is shown in the following equation [8]:

$$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O \quad (3)$$

$$MnO_4^- + 2H_2O + 3e^- \rightarrow MnO_2 + 4OH^- \quad (4)$$

Value of Power Density (mW/cm^2)

Based on the maximum value of current and potential difference, the efficiency value of Power Density (mW/cm^2) can be calculated. The resulting Power Density value was 982 mW/cm^2. Based on Permana et al. (2015) research, which used glucose and yeast as a microorganism produced a Power Density value of 4.48 x 10^{-3} W/m^2 [8]. The results obtained indicated that the value of Power Density using glucose substrate from the red algae *E. cottonii* was higher because, based on the study results from Ullah and Zeshan (2020), the type of substrate used affects the performance of the MFC [25]. In addition, the substrate concentration also affected the resulting power density [21].

CONCLUSION

Based on the research results, it is known that using red algae (*E. cottonii*) extract as a subtract in the MFC system can produce electrical energy. Its Power Density values are 982 mW/cm^2, while the maximum current and potential difference value is 0.52 mA and 370 mV, respectively.

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