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Electrical Energy Production with Microbial Fuel Cell (MFC) Technology on *Gracilaria verrucosa* Substrate

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ABSTRACT

Microbial Fuel Cell (MFC) is a renewable technology based on electrochemistry that has the ability to convert chemical energy into electrical energy through microbial metabolic processes. This study aims to determine the effect of adding an electrolyte solution to the production of electricity generated using the substrate *Gracilaria verrucosa* and the microbe *Saccharomyces cerevisiae*. The method used in this study uses a double chamber consisting of anode and cathode chambers connected through a proton exchange membrane (PEM) in the form of Nafion 117. The results showed that the addition of 0.2 M KMnO4 electrolyte solution produced current, voltage and power density respectively. of 0.74 mA; 580 mV and 2187 mW/cm^2 higher than the addition of 0.2 M $K_3Fe(CN)_6$ electrolyte solution resulted in a current, voltage and power density of 0.69 mA, respectively; 450 mV and 1582.16 mW/cm^2. The MFC system using *Gracilaria verrucosa* as a substrate has the potential to generate electrical energy.

Keywords: Microbial Fuel Cell (MFC), Electrical energy, *Gracilaria verrucosa*, *Saccharomyces cerevisiae*

INTRODUCTION

An increase in the number and economic activity of the population to meet their daily needs can lead to an increase in the need for electrical energy. However, the energy used by society and industry is still in the form of conventional energy, namely fossil fuels. Fossil fuels are carbon-based energy sources that are non-renewable. Excessive use of fossil fuels can increase air pollution and the greenhouse effect from carbon dioxide (CO_2) emissions produced(Perera, 2018). Therefore, alternative energy sources are needed to reduce the use of fossil fuels, namely energy sources that are derived from cell-based hydrogen fuels. microbial fuel cell (MFC).

The MFC system has advantages over fuel cells. This is because the electrical energy generated from MFC comes from the degradation of organic waste and biomass and uses a microorganism catalyst which is cheaper than a platinum fuel cell catalyst, which is quite expensive(Pratama, 2020). The MFC system is an electrical energy generation system that has the ability to convert chemical energy into electrical energy(Vera Natalia Ginting et al., 2019). The MFC system will oxidize the substrate in the form of organic material at the anode which will produce CO_2, protons (H+) and electrons. The resulting electrons are then given to NAD^+ which will be reduced to NADH. NADH is a coenzyme that acts as an electron carrier in cellular metabolic processes. Methylene blue in the anode chamber will penetrate the cell membrane and win P electrons carried by NADH. Methylene blue is reduced to colorless MBH_2

and diffuses outside the cell membrane to release electrons at the electrodes in electron transfer. MB then diffuses and delivers electrons to the electrode surface without using yeast cells to be attached to the electrode surface. Next, electrons are transferred to the cathode surface via copper wires and protons diffuse to the cathode compartment through the proton exchange membrane(Yuan et al., 2020). Electrical energy is obtained based on the speed with which electrons flow through the circuit and the difference in electrochemical potential across the electrodes(Muftiana et al., 2018).

Factors that can affect the MFC system in generating electrical energy are the substrate and the electrolyte solution. The use of substrates in the MFC system is used to generate electricity and a source of nutrition for microbes to carry out cell metabolism(Yuan et al., 2020). The substrate used in the MFC system can be obtained from simple pure components and complex components. Simple pure components consist of one component, namely acetate, butyrate, propionate(Chae et al., 2009), sucrose(Behera & Ghangrekar, 2009), starch(Niessen et al., 2004), glucose(Sayed et al., 2012) dan cellulose(Ming-Ju Chen, Kreuter, 1996). The complex component in the MFC system consists of several components, namely molasses waste(Zhong et al., 2011), paper recycling plant(Huang & Logan, 2008), wastewater from food processing(Mansoorian et al., 2013), brewery wastewater(Feng et al., 2008) dan algae(Arun et al., 2020). The content contained in red algae (*Gracilaria verrucosa*) consists of a protein content of 4.609%, a fat content of 3.322%, an ash content of 19.575% and a carbohydrate content of 72.495%(Farid et al., 2013).

The use of solutions in the MFC system can also affect the amount of electrical energy produced. An electrolyte solution is a solution that can conduct electricity. Electrolyte solution $KMnO_4$ and $K_3Fe(CN)_6$ are oxidizing agents used as electron acceptors which have standard reduction potentials of 1700 mV and 360 mV, respectively. Protons and electrons in the anode space will reduce Mn^{7+} to Mn^{4+} from solution $KMnO_4$ and reduce Fe^{3+} to Fe^{2+} from solution $K_3Fe(CN)_6$. The meeting between protons and electrons will cause a potential difference between the ends of the electrodes at the cathode and anode(Muftiana et al., 2018). Based on(Yuan et al., 2020), electrical energy research based on MFC with glucose as a substrate using *Saccharomyces cerevisiae* and electrolyte solution $K_3Fe(CN)_6$ resulted in a maximum power density of 5,2 ± 0,5 mW/cm^2. Based on the results of the study(Permana et al., 2015) electrical energy based on MFC was generated using $KMnO_4$ electrolyte solution which produced a current of 5,5x10^{-5} A, a potential of 886 mV and a power density of 4,48x10^{-3} mW/cm^2.

In this paper, the application of MFC technology on the substrate of red algae (*Gracilaria verrucosa*) to produce electrical energy. It is hypothesized that there is an effect of using distilled water, electrolyte solution $KMnO_4$ 0.2 M and $K_3Fe(CN)_6$ 0.2 M on the electricity generated. Furthermore, yeast growth in the anode compartment indicated the performance of the yeast-MFC system.

MATERIALS & METHODS

Materials

Materials used in this study include red algae (*Gracilaria verrucosa*), aquadest (H_2O), 3,5-Dinitro salicylic acid (DNS), yeast extract, graphite electrodes, hydrogen peroxide (H_2O_2) 3%, hydrochloric acid (HCl) 1 M, sulfuric acid (H_2SO_4) 1 M, 7% and 72%, potassium dihydrogen phosphate (KH_2PO_4), potassium permanganate ($KMnO_4$) 0.2 M, potassium hexacyanoferate (III) ($K_3(Fe(CN)_6$) 0.2 M, methylene blue, *Nafion* 117, sodium hydroxide (NaOH) 1 M and peptone. The instruments used in this study are Visible Spectrophotometer (Thermo scientific Genesys 20), UV-Vis Spectrophotometer (Varian Cary 50), Refractometer, Gas chromatography–mass spectrometry (GC-MS) (Thermo scientific

ISQ 7000) and Digital Multimeter (ANENG A830L).

Methods

MFC Construction

The MFC tool is made of acrylic with a size of 10 x 10 x 10 cm. The MFC reactor consists of two compartments, namely the anode chamber and the cathode chamber which are separated by PEM, namely Nafion 117. Each compartment holds 1 L which has a hole with a diameter of 3.5 cm in each compartment and paired PEM as a proton transfer site. Furthermore, 5 graphite electrodes are attached to each compartment and connected to a series of wires on a modified digital multimeter (Yuan et al., 2020)modified.

Electrode and PEM Preparation

Electrode preparation was done by immersing graphite electrode rods with hydrochloric acid (HCl) 1 M for 1 x 24 hours, and then rinsed with distilled aquadest (H_2O). After that, the graphite electrode was immersed again with sodium hydroxide (NaOH) 1 M for 1 x 24 hours. Next, the graphite electrode was rinsed with distilled aquadest (H_2O) until neutral(Baharuddin et al., 2020) Meanwhile, the PEM preparation was carried out by means of a membrane in the form of *Nafion* 117 heated using aquadest (H_2O) at 80˚C for 1 hour, then heated using H_2O_2 3% 80˚C for 1 hour and rinsed with aquadest (H_2O). Furthermore, the Nafion 117 membrane was reheated with H_2SO_4 1 M solution for 1 hour and washed with aquadest (H_2O) 3 times. The membrane was soaked with aquadest until it was ready to use. The membrane was aerated before it was applied to the MFC reactor(Utami et al., 2020).

Preparation of Red Algae (Gracilaria verrucosa)

Gracilaria verrucosa was washed thoroughly with running water and cut into small pieces and dried. The red algae were ground using a grinding machine to become a fine powder. Next, the red algae were sieved using a sieve with a size of 100 mesh.

Determination of ADF, NDF, Lignin, Cellulose and Hemicellulose Contents in Gracilaria verrucosa

a. *Determination of ADF*

As much as 0.3 g of G. verrucosa powder (a) was put into a test tube and added 40 mL of ADF solution and then closed tightly. Next, the mixture is refluxed in boiling water for 1 hour. After that, it was filtered with sintered glass of known weight (b) while sucked with a vacuum pump. Next, the residue was washed with boiling water of approximately 100 mL until the foam disappeared and 50 mL of alcohol. The residue was dried in an oven at 100 C for 8 hours. Furthermore, it is cooled in a desiccator for approximately 30 minutes and then weighed (c)(Peternakan & Hasanuddin, 2014).

$$\text{ADF Level} = \frac{c-b}{\text{sample weight (a)}} \text{ x } 100\% \quad (1)$$

b. *Determination of NDF*

A total of 0.2 g of G. verrucosa powder (a) was put into a test tube and 25 mL of NDS solution was added and then tightly closed. Next, the mixture is refluxed in boiling water for 1 hour. After that, it was filtered with sintered glass of known weight (b) while sucked with a vacuum pump. Next, the residue was washed with boiling water of approximately 100 mL until the foam disappeared and 50 mL of alcohol. The residue was dried in an oven at 100 C for 8 hours. Furthermore, it is cooled in a desiccator for approximately 30 minutes and then weighed (c) (Karim, 2014)(Peternakan & Hasanuddin, 2014).

$$\text{NDF Level} = \frac{c-b}{\text{sample weight (a)}} \text{ x } 100\% \quad (2)$$

c. *Determination of Cellulose and Lignin Content*

Sintered glass containing ADF was placed on a petridisk and added 20 mL of 72% H_2SO_4. Next, sucked with a vacuum pump while rinsing with enough hot water. After that, it was dried in an oven at 100 C for 8 hours. Furthermore, it is cooled in a desiccator and then

weighed (d). Next, the residue is heated using a furnace to a temperature of 500 C for 2 hours and allowed to cool slightly then put in a desiccator for 30 minutes and weighed (e)(Peternakan & Hasanuddin, 2014).

$$\text{Lignin Content} = \frac{d-e}{\text{sample weight (a)}} \text{ x } 100\% \quad (3)$$

%Cellulose = % ADF-% Insoluble ash-lignin
% Hemicellulose = % NDF- % ADF

Cellulose Hydrolysis

A total of 9 g of G. verrucosa added 180 mL H_2SO_4 7%. Next, the mixture was heated at 100˚C for 1 hour while stirring and filtered(Armawan Sandi et al., 2016).

Measurement of Reducing Sugar from Hydrolysis of Gracilaria verrucosa

A total of 2 mL of the hydrolyzed G. verrucosa added 2 mL of DNS reagent, then mixed and heated at 100˚C for 10 minutes. The measurement of reducing sugar was carried out with a spectrophotometer UV-Vis with a wavelength of 540 nm. Measurement of reducing sugar is based on the standard glucose curve(Agustini & Febrian, 2019).

MFC Experiment

A total of 40 mL glucose hydrolyzed *Gracilaria verrucosa*, 5 g peptone, 5 g yeast extract, 5 g KH_2PO_4, and 2 mL MB in 800 mL were added into the anolyte chamber. The pH value of the anolyte was initially adjusted to 7.0 by NaOH 5 M. The anolyte solution was autoclaved at 121 °C for 15 min separately and exposed under UV light inside a laminar hood before use. Then 1 g of dry yeast was mixed with 20 g of glucose in 50 mL and stored for 1th hour at 37˚C. After that, the yeast solution was put into the anolyte and stirred. Then 800 mL of $KMnO_4$ 0.2 M was put into the catholyte chamber. After that, the chamber cover was installed and connected to a multimeter to measure the current and voltage generated every 4th hours for 48th hours. For $K_3(Fe(CN)_6)$ 0,2 M electrolyte solution, the same thing was done in the previous description, where $KMnO_4$ 0.2 M solution was replaced with $K_3(Fe(CN)_6)$ 0.2 M solution and without the addition of an electrolyte solution(Yuan et al., 2020) modified.

The power density value was obtained from the current and voltage strength data which was calculated using the equation in formula 1:

$$Power\ Density\ \left(\frac{mW^2}{m}\right) = \frac{V\ x\ I}{A} \quad (4)$$

The concentration of yeast in the MFC solution was determined by measuring the optical density using a Visible spectrophotometer with a maximum wavelength of 600 nm.

RESULT AND DISCUSSION

Gracilaria verrucosa Substrate in the MFC System

The use of substrates in the MFC system can affect the electricity generated. The substrate in the MFC system is used as a source of nutrition for microbes in carrying out cell metabolism(Permana et al., 2015; Yuan et al., 2020). The results of the analysis of the content of ADF, NDF, insoluble ash, cellulose, hemicellulose and lignin in *Gracilaria verrucosa* is shown in Table 1.

Table 1. The content of ADF, NDF, insoluble ash, cellulose, hemicellulose and lignin in *Gracilaria verrucosa*.

Types of Marine Algae	Content (%)					
	ADF	NDF	Lignin	Insoluble ash	Cellulose	Hemicellulose
Gracilaria verrucosa	16,93	21,18	1,87	11,70	3,36	4,25

The levels of lignin and cellulose obtained in this study were different from those obtained by(Artikel, 2016), Gracilaria verrucosa powder contains lignin and cellulose content of 3.33% and 4.48%, respectively. (*Ika Septiany Program Pascasarjana*, 2013)the lignin and cellulose content of Gracilaria verrucosa were 8.76% and 8.23%, respectively. This may be due to the different uptake sites for *Gracilaria verrucosa* resulting in different levels of lignin and cellulose. The results of the measurement of glucose levels of *Gracilaria verrucosa* from hydrolysis are shown in Table 2.

Table 2. *Gracilaria verrucosa* glucose levels from hydrolysis

No	Substrate Type	Glucose Level (mg/mL)
1	*Gracilaria verrucosa*	0,0745

The Growth Curve Measurement of Saccharomyces cerevisiae Yeast

The suitability of the use of microbes with certain substrates plays a very important role in generating electricity. The amount of electrical energy obtained in the MFC system is influenced by the metabolic rate carried out by microbes. Therefore, the use of different microbes will produce different electrical energy. In this study, the growth of Saccharomyces cerevisiae was carried out by measuring the OD at 600 nm every 4 hours for 48 hours to see the ability of cells to degrade organic matter. The growth curve of Saccharomyces cerevisiae using *Gracilaria verrucosa* as a substrate is shown in Figure 1.

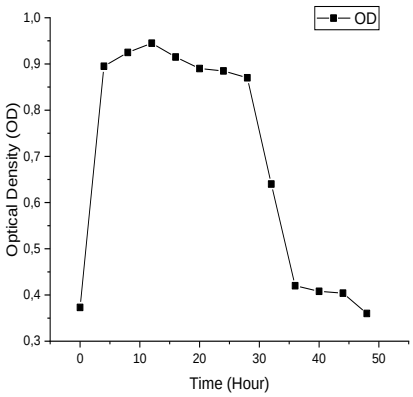

Figure 1. Growth Curves of *Saccharomyces cerevisiae*

Based on Figure 1, the exponential phase of the growth of Saccharomyces cerevisiae was produced at 0 to 12 hours. The exponential phase in Saccharomyces cerevisiae can maintain its growth by producing ATP through the fermentation process(Olivares-Marin et al., 2018). If the OD value is greater, it is possible that there will be more cells in the reactor. The increasing number of cells allows for more protons and electrons that can be generated from metabolic processes so that electrical energy will be even greater(Novitasari, 2011).

Effect of Addition of Electrolyte Solution $KMnO_4$ 0,2 M on Current and Voltage Values on *Gracilaria verrucosa* Substrate

The measurement of current and voltage with *Gracilaria verrucosa* substrate were carried out every 4 hours for 48 hours using *Saccharomyces cerevisiae* with and without a combination of electrolyte solution $KMnO_4$ 0,2 M as shown in Figure 2.

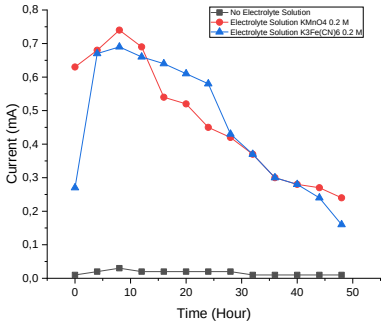

Figure 2. Effect of Addition of electrolyte solution $KMnO_4$ 0,2 M and $K_3Fe(CN)_6$ 0,2 M to Current Value on *Gracilaria verrucosa* Substrate.

Based on Figure 2, the maximum current generated on the substrate Gracilaria verrucosa without the addition of electrolyte solution was obtained at the 8th hour of 0.03 mA. This is in accordance with the growth of Saccharomyces cerevisiae which experienced an exponential phase at 0 to 12 hours. This is in accordance with research(Zahara, 2011) the effect of the exponential phase on the MFC system is that Saccharomyces cerevisiae cells can produce more electricity due to division and an increase in the number of cells. The maximum current produced without a combination of electrolyte solution is lower than the maximum current produced with the addition of an electrolyte solution. The maximum current produced with *Gracilaria verrucosa* substrate with a combination of $KMnO_4$ 0,2 M electrolyte solution was obtained at the 8th hour of 0.74 mA and electrolyte solution $K_3Fe(CN)_6$ 0,2 M was obtained at the 8th hour of 0.69 mA. This may be because the addition of an electrolyte solution in the MFC system can increase the electric current generated(Rahmaniah et al., 2020). The maximum current generated by

the addition of electrolyte solution $KMnO_4$ 0,2 M is greater than the addition of electrolyte solution $K_3Fe(CN)_6$ 0,2 M. This is because $KMnO_4$ has a standard reduction potential value of 1300 mV which is higher than $K_3Fe(CN)_6$ of 360 mV(Ardi, 2020).

The current value with the addition of electrolyte solution $KMnO_4$ 0,2 M and $K_3Fe(CN)_6$ 0,2 M at 12 to 48 hours decreased continuously. This may be due to the decrease in glucose levels in the anode space with increasing time. This causes a decrease in the speed of cell metabolism so that the resulting current decreases. In addition, the decrease in the current value was also due to *Saccharomyces cerevisiae* having entered a stationary phase where the number of live cells was the same as the number of dead cells(Arbianti et al., 2013).

The maximum current value generated in this study with the addition of electrolyte solution $KMnO_4$ 0,2 M dan $K_3Fe(CN)_6$ 0.2 M was higher than the research conducted(Pengaruh et al., 2019), rotten tomato substrate was 0.38 mA. This is due to the addition of rice field mud which contains nitrate which can become an electron acceptor in the anode compartment and causes low electricity production in the MFC system as a result of the denitrification process(Pengaruh et al., 2019).

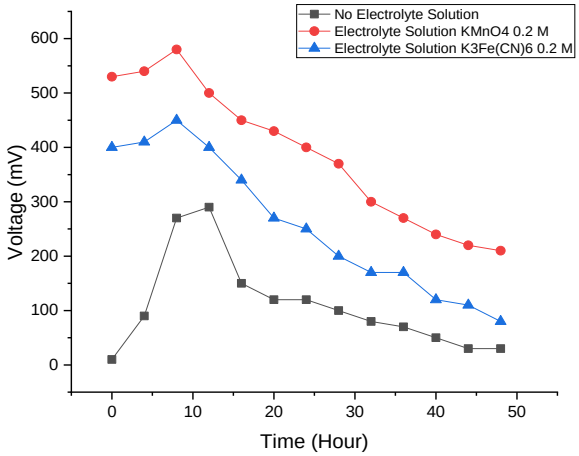

Figure 3. Effect of Addition of electrolyte solution $KMnO_4$ 0,2 M and $K_3Fe(CN)_6$ 0,2 M to Voltage Value on *Gracilaria verrucosa* Substrate

Based on Figure 3, the maximum voltage produced on the Gracilaria verrucosa substrate without the addition of electrolyte solution was obtained at the 12th hour of 290 mV The voltage value obtained in this study is higher than the voltage value obtained(Ardi, 2020) by 91.4 mV. This is because the substrate used is different from the research conducted, namely sago stalks, so it takes a longer time to break down molecules from the polysaccharide group into simple molecules(Ismawati et al., 2015). The maximum voltage value without the addition of electrolyte solution is lower than the maximum voltage value with the addition of electrolyte solution $KMnO_4$ 0.2 M and $K_3Fe(CN)_6$ 0.2 M.

The maximum voltage value with Gracilaria verrucosa substrate with the addition of electrolyte sution $KMnO_4$ 0,2 M was obtained at the 8th hour of 580 mV and $K_3Fe(CN)_6$ 0.2 M electrolyte solution was obtained at the 8th hour of 450 mV. The decrease in the value of the voltage occurred at the 12th to 48th hours. The decrease in the voltage value may be due to the decomposition of microbial food nutrients during the MFC process and an increase in side products in the form of CO_2 which causes a toxic effect on cells so that the pH will decrease and result in a decrease in

microbial metabolism(Fitriani et al., 2017). The maximum voltage value produced in this study with the addition of electrolyte solution $KMnO_4$ 0.2 M and $K_3Fe(CN)_6$ 0,2 M was higher than the research conducted(Öztürk & Onat, 2017) with molasses substrate without a mediator of 375 mV. This may be because this research uses the help of an electron mediator in the form of methylene blue which can increase the voltage value in the MFC system(AP et al., 2018). Methylene blue has the ability to diffuse and send electrons to the electrode surface without requiring Saccharomyces cerevisiae cells to attach to the electrode surface(Yuan et al., 2020).

Based on the current and voltage values obtained with *Gracilaria verrucosa* substrate, the current and voltage values with the addition of electrolyte solution $KMnO_4$ 0,2 M were higher than the addition of electrolyte solution $K_3Fe(CN)_6$ 0,2 M.

Power Density

Based on the maximum value of current and voltage on the substrate *Gracilaria verrucosa* with and without the addition of eelctrolyte solution $KMnO_4$ 0.2 M and $K_3Fe(CN)_6$ 0.2 M, the power density (mW/cm^2) is obtained as shown in Figure 4.

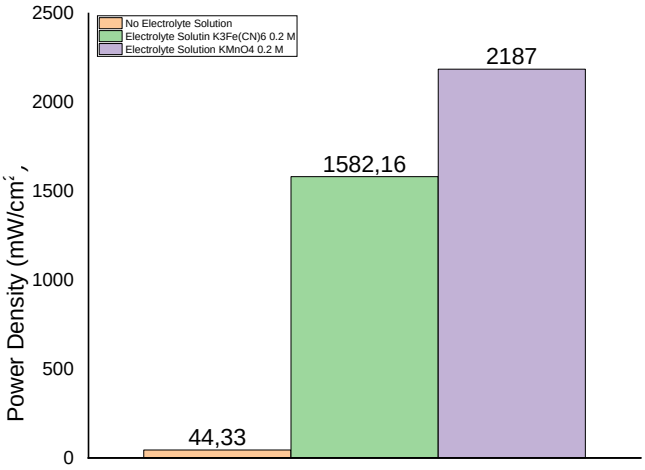

Figure 4. Power Density (mW/cm^2) Comparison with *Gracilaria verrucosa* Substrate with and without Electrolyte Solution

Based on Figure 4, the power density (mW/cm^2) on *Gracilaria verrucosa* substrate with the highest addition of electrolyte solution was obtained by adding electrolyte solution $KMnO_4$ 0,2 M of 2187 mW/cm^2. The power density with the addition of electrolyte solution obtained was higher than that obtained in(Toczyłowska-Mamińska et al., 2018) with cellulose substrates of 44 mW/cm^2 and study(Ullah & Zeshan, 2020) sucrose substrates of 64 mW/cm^2. This may be due to the addition of electron mediators which play a role in increasing the performance of the MFC in generating electricity.

CONCLUSION

In this study, the production of electrical energy with an electrochemical-based MFC system was carried out.. The addition of electrolyte solution $KMnO_4$ 0.2 produced a current, voltage and power density of 0.74 mA, 580 mV and 2187 mW/cm^2 higher than the addition of electrolyte solution $K_3Fe(CN)_6$ 0.2 M resulted in a current, voltage and power density of 0.69 mA, 450 mV and 1582.16 mW/cm^2.

Declaration by Authors

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