Development of Microalgae-microbial Fuel Cell (MmFC) Technology using Microalgae Consortium of Chlorella vulgaris and Spirulina Platensis

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Development of Microalgae-microbial Fuel Cell (MmFC) Technology using Microalgae Consortium of Chlorella vulgaris and Spirulina Platensis

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Abstract: Microalgae-microbial Fuel Cell (MmFC) is a type of Microbial Fuel Cell (MFC) that is integrated with the microalgae cultivation system. This device is capable of supplying electron acceptor (oxygen) that are naturally produced by microalgae that are not found in common MFCs. In this study, the development of MmFC was carried out using a consortium of microalgae consisting of *Chlorella vulgaris* and *Spirulina vulgaris* as the most studied microalgae and have the potential to support each other in terms of growth. *Spirulina* is known to provide compounds that support the growth of microalgae such as vitamins and inorganic nutrients. This research began with a pre-culture in F/2 medium using various ratios of *Chlorella:Spirulina* consortium composition ratios (1:0, 0:1, 1:1, 2:1, 3:2). The consortium at 3:2 ratio produced highest biomass and growth rate of 3.94 g/L at the 222nd hour and 0.021/hour at the 110th hour. This consortium also produced the highest oxygen content that could be used as an electron acceptor, which was 6.77 mg/L at 192 hours. The consortium was then cultured in a 500 mL MmFC dual-chamber device equipped with a Nafion 117 membrane and carbon (graphite) rod electrodes. The cathode compartment is used for the cultivation of the microalgae consortium, while the anode compartment is filled with artificial tempe (fermented soybean cake) wastewater containing indigenous microbes capable of degrading organic compounds. After 49 hours of operation, the highest voltage and electric power obtained are 437.1 mV and 1.911 mW, respectively, at 24 hours with anode surface area of 59.06 cm^2.

Keywords: Microalgae-microbial Fuel Cell; Bioelectricity; Microalgae Consortium; *Chlorella vulgaris; Spirulina platensis*

1. Introduction

Microbial Fuel Cell (MFC) is a type of Fuel Cell (FC) that uses active microorganisms to produce bioelectricity. This system generates electric current with the help of biocatalytic redox reactions by microorganisms[1)]. In the MFC compartment, microorganisms will degrade organic compounds by releasing electrons and proton[2)]. Protons will be transferred directly to the cathode while electrons will be transferred via external circuit, where this process plays a role in generating electric current. When protons and electrons reach the cathode, they reduce the available oxygen (O_2) and form water molecules[3)]. Direct conversion of substrate to energy in MFC results in high efficiency[4)].

One of the important factors affecting electricity production by MFC is electron acceptor[5)]. In general, the use of oxygen as an electron acceptor in MFC is preferred due to its easy accessibility, intense oxidation potential, and does not produce toxic chemical waste (only produces water as the final product)[6)]. Several previous studies provide oxygen directly into MFC, but this process consumes a large amount of energy[5)]. In other studies, an air-cathode has been developed, where the cathode is made with one side interacting directly with the electrolyte fluid and the other side interacting with the air[7)] so that oxygen in the air can be directly utilized. Nonetheless, the use of air-cathode still has some limitations related to air contact on the cathode surface, thus possibly requiring the use of a costly catalyst[5)]. Considering the importance of oxygen in MFC, the method of supplying oxygen is an important aspect of its

design. Several studies have demonstrated the ability of microalgae to integrate with MFC systems based on its photoautotroph nature, where these microalgae can act as in situ O_2 producers to facilitate reactions in the cathode compartment[1].

Microalgae are a group of photosynthetic microorganisms related to their ability to harness the energy of sunlight and absorb carbon dioxide (CO_2) from the environment for photosynthesis[8]. *Chlorella vulgaris* and *Spirulina platensis* are two types of microalgae that have the potential to be used in MmFC and are the most widely used in research worldwide[4]. According to research conducted by Fu et al. on Photosynthetic Microbial Cell (PMC) using *Spirulina platensis*, the resulting power density of 6.6 mW/m^2 while based on research by Wu et al. in MmFC using *Chlorella vulgaris* with a tubular photobioreactor circuit at the cathode, the resulting power density of 21.4 mW/m^2[1]. This shows that these types of microalgae can be used in MmFC. When grown together in the same culture, these microalgae can have symbiotic bond. *Cyanobacteria*, including *Spirulina platensis* can provide compounds that support growth such as vitamins and inorganic nutrients (Fe, CO_2, NH_4^+, NO_3, or PO_4^{3-})[9]. The use of *cyanobacteria* is also very promising because of their fast growth ability[10]. On the other hand, microalgae can produce an envelope layer for the formation of microalgae cell aggregation to form a biofilm that enhances the interaction between those two[11]. This interaction demonstrates the potential of the combination of the microalgae *Chlorella vulgaris* and *Spirulina platensis* in optimizing culture growth to develop MmFC.

There are now numerous studies that combine the benefits of bioelectricity production and wastewater recovery in the MFC. Generally, wastewater already contains indigenous bacteria which can be directly used as a degrading agent. Indonesia is one of the largest soybean consumers in the world, around 90% of which is used as an ingredient of soybean cake and tofu. In the soybean processing industry, wastewater generated will be an environmental problem if disposed of directly because it will deteriorate very quickly due to the high-water content and nutrients for bacterial growth[12]. One of the studies on MFC conducted by Utami et al. (2018)[13] in Bioprocess Engineering, University of Indonesia, taking tempe (fermented soybean cake) wastewater as a substrate in the MFC series. This research was conducted in a single-chamber (SC) MFC using graphite electrodes with an active surface area of 127.75 cm^2 at the anode. The cathode is made to be in direct contact with the outside air (air-cathode). This 50-hour study resulted in a voltage of 291.1 mV and electrical energy of 66.33 mW/m^2 at 1% culture concentration[13]. The research became the basis for the development carried out in this study. Thus, tempe wastewater was used in this study, focuses on developing microalgae consortium utilization.

Other research related to microalgae and wastewater utilization for MmFC conducted by Huarachi-Olivera et al. (2018)[14]. The research used cocoa industrial wastewater with a consortium of anaerobic microbes in the anode compartment and *Chlorella vulgaris* in the cathode compartment. The configuration used is a double-chamber (DC) MmFC with a volume of 1L in each compartment and separated by PEM Nafion 117. The electrode used is a graphite slab with a surface area of 58.2 cm^2 and a 1.5 W LED lamp is used as a resistor. The cathode compartment is left open for CO_2 fixation by microalgae. With that configuration, 23.17 mW/m^2 of electrical energy is generated at the beginning of operation and continues to increase to 327.67 mW/m^2 on the 32nd day with a voltage of 224 mV to 954 mV from the beginning of operation to the 32nd day. The growth of microalgae at the beginning of the operation was 3.76 mg/mL while on the 32nd day it was 5.2 mg/mL. Rosyadi et al. (2017)[15] previously also conducted research using tempe wastewater as oxidation source and *Cladophora* macroalgae as a biocatalyst. Tempe wastewater from the industry was previously incubated for 7 days to increase the concentration of the bacterial consortium in it. For *Cladophora*, before being used in MmFC, it was first grown in distilled water and placed in a place exposed to light for 3 weeks. In the study, a DC MmFC configuration with a cation exchange membrane and graphite electrode was used. The anode compartment uses 400 mL of liquid tempe waste with the addition of 200 mL of 1M glucose, while the cathode compartment uses *Cladophora* with the addition of 600 mL of distilled water. Based on the research conducted, a maximum voltage of 320 mV at 60 grams of algae content was obtained. The maximum current measured is 5.9 A, and the electrical energy produced is 1293.151 W/m^2.

This study examined bioelectricity generated by DC MmFC with artificial tempe wastewater and microalgae consortium of *Chlorella vulgaris* and *Spirulina platensis*. The growth characteristics of microalgae *Chlorella vulgaris*, *Spirulina platensis*, and its consortium was also investigated.

2. Materials and Methods

The materials used include materials for microalgae cultivation, and materials for MmFC operations. The materials used in microalgae cultivation are *Chlorella vulgaris* from Malang, Indonesia; *Spirulina platensis* from Grobogan, Indonesia; Guillard F/2 growth medium from Xwarpshop Store, and aquadest. Materials for MmFC operation are cultivation materials plus soybeans (*Glycine max*) from Depok market; 99,9% graphite electrode cylinder rod from Ninetynine Store; HCl and NaOH for electrode preparation; DuPont Proton Exchange Membrane (PEM) Nafion 117; H_2SO_4 and H_2O_2 for membrane preparation. In this study, a modified MmFC reactor from CV. Pugar Mandiri was used with two 500 mL chambers connected with a membrane.

2.1 Microalgae and Consortium Pre-culture

Growth observation before operation of MmFC was carried out to examine the growth rate achieved by pure microalgae cultures of *Chlorella vulgaris* and *Spirulina platensis* compared to the microalgae consortium. Microalgae *Chlorella:Spirulina* was grown at different ratios of 1:0, 0:1, 1:1, 3:2, and 2:1 in 250 mL culture. Guillard F/2 medium was used for cultivation. Based on the literature, the composition of the Guillard F/2 medium was dominated by the content of $NaNO_3$ as a nitrogen source[16]. The variation of the microalgae consortium was made by combining microalgae at an OD540 about 0.3 based on the volume ratio.

2.2 MmFC Reactor Preparation

Electrode and membrane used in this study is a carbon rod (graphite) and PEM Nafion 117. Electrode was pretreated using 1M HCl and 1M NaOH to increase the active surface area and its adsorption capacity. Meanwhile, the membrane was pretreated in 3% H_2O_2 and 1M H_2SO_4 at 80°C. The use of H_2O_2 aims to remove organic compounds from the membrane whilst H_2SO_4 aims to activate the membrane by increasing the number of water molecules per sulfonate group[17]. Electrode and membrane are immersed in distilled water until it is used.

Anode and cathode compartment was filled with artificial tempe wastewater and microalgae culture, respectively. In tempe wastewater process-making, soybeans (*Glycine max*) were boiled in water for 15 minutes at a ratio that followed the soaking process for soybeans in the tempe-making process, which was a ratio of 3:5 (w/v). The water is then incubated in an incubator at 37°C for 1 week because the microbes that grow in it are quite stable and the organic substrate content in it is still quite high[18]. After incubation, the OD486 value is determined using the procedure described in in the study of Utami (2014)[19] which involved measuring the optical density of microbes in tempe wastewater. The preparation of the microalgae consortium for MmFC was carried out with the same procedure as for pre-culture.

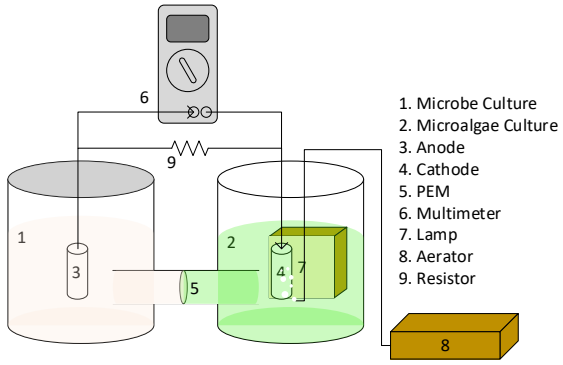

Fig. 1: Microalgae-microbial Fuel Cell Configuration

The electrodes in this study had a surface area of 29.5 cm^2 per rod. In this study, two rods were used for each compartment so that the surface area of the anode and cathode were 59 cm^2 each. A copper wire with alligator clips is used to connect the electrodes to a multimeter with a resistor (100 ohms) used in the electrical circuit.

Nafion 117 membrane is placed in the hole between the two compartments as a place for proton transfer from the anode compartment to the cathode compartment while limiting the transfer of oxygen to the anode compartment which can reduce the reduction potential at the cathode. The membrane in this study has a cross-sectional area of 2.4 cm^2.

In the cathode compartment where microalgae grow, aeration is provided to homogenize the culture and prevent biomass sedimentation. Lamps was also provided for microalgae lighting (3000 lux). Light is important limiting factor as long as nutrients are available[20]. Figure 1 shows MmFC configuration used in this experiment.

2.3 MmFC Operation and Data Calculation

The MmFC device was operated for 49 hours with continuous aeration and lighting for microalgae. During the operation of the MmFC, the performance parameters of the MmFC were observed including the growth rate of microalgae through optical density readings and the value of the electrical voltage generated through multimeter readings.

Table 1. Calibration Curve Equation for Microalgae Consortium (OD540 vs. X)

Chlorella:Spirulina	Equation
1:1	$X = 2.7474(OD) - 0.0584$
2:1	$X = 1.5653(OD) - 0.1885$
3:2	$X = 1.9220(OD) - 0.1050$

Microalgae growth was determined by taking OD540 microalgae data and the value was converted to biomass dry weight (X) using a calibration curve equation (OD540 vs. X) as in Table 1. Microalgae dry weight data was used to see the growth rate. The equation used to calculate the specific growth rate of microalgae is the Monod equation which can be written as follows[21].

$$\mu = \frac{1}{x}\frac{dX}{dt} \quad (1)$$

Where μ is the specific growth rate ($hour^{-1}$), X is the dry weight of cells (g/dm^3), and t is time (hours).

Electrical data taken in the MmFC circuit is the value of the electric voltage (P). The value of the electric voltage obtained during the operation of the MmFC will later be processed into the value of electrical energy (power density) which is the amount of energy produced per anode surface area. Power density data processing is carried out based on the following calculations [13].

$$P = \frac{V \times I}{A} \text{ or } P = \frac{V^2}{R \times A} \quad (2)$$

Where P is the power density (mW/m^2), V is the electric

voltage (volt), I is the electric current (ampere), A is the anode surface area (m^2), and R is the resistance (ohm).

3. Results and Discussion

MmFC works with reduction and oxidation potentials as a driving force, thus the level of redox potential plays a very important role in determining the electrical energy output of MmFC. The oxidation reactions involved and their reduction potentials if the organic compound is assumed to be glucose are as follows (E^0_{red}=4,30 V)[22]:

$$CO_2 + 4H^+ + 4e^- \rightarrow [CH_2O]glucose + H_2O \quad (3)$$

The reaction of electron reduction by oxygen produced by microalgae in the cathode compartment are as follows (E^0_{red}=1,23 V)[23]:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (4)$$

Fuel cell is a type of battery in which an oxidizing agent and a reducing agent are continuously added. Therefore, as long as the reactants are continuously supplied, energy will continue to be produced[24]. In MmFC, the oxidizing agent is oxygen, which is provided continuously during the growth of microalgae, while the reducing agent is an organic compound in tempeh wastewater. Therefore, the potential for microalgae culture of *Chlorella vulgaris* and *Spirulina platensis* and their consortium in MmFC will be examined.

3.1 Increasing Biomass Production of Microalgae Consortium

The optical density of microalgae was recorded for 222 hours of cultivation. In general, the density of microalgae culture increased starting on the 7th day or 168th hour. This shows that the growth of microalgae has entered an exponential phase with a lag phase of between 5-6 days. The consortium of microalgae (KCS) with a ratio of *Chlorella:Spirulina* 3:2 increased the most to reach 3.94 g/L at 222 hours, followed by a consortium with a ratio of 1:1 which was 3.86 g/L; the 2:1 ratio consortium is 2.01 g/L; *Chlorella vulgaris* is 0.76 g/L; and *Spirulina platensis* which is 0.55 g/L.

In Figure 2, it can be observed that the growth rates of pure *Chlorella vulgaris* and *Spirulina platensis* tend to be lower than those of the consortium cultures. These results indicate a tendency where when *Chlorella vulgaris* is added with *Spirulina platensis* to a ratio of 2:1 and 3:2, the growth will increase. However, when it is increased again until the composition is the same, as in 1:1, the biomass produced will be less. This can occur due to the potential competition between the two microalgae species at a certain ratio as stated by Ji et al. (2017)[25] that in addition to a beneficial symbiosis, these two species also experience competition, one of which is the availability of CO_2. Microalgae typically absorb dissolved CO2 into their cells. A higher concentration of dissolved CO2 means that the cells can use more carbon[26].

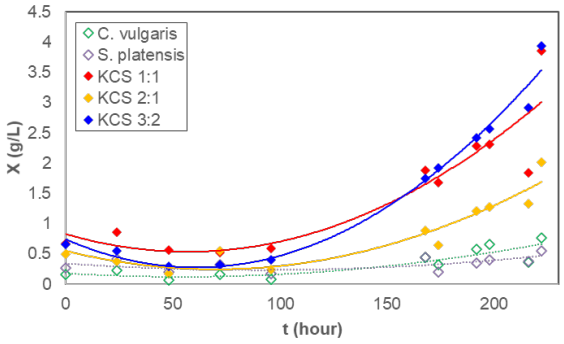

Fig. 2: Biomass Growth Curve of Microalgae and Consortium

In Figure 3, it was observed that the growth rate increased between the 50th hour to the 100th hour and then decreased slightly. This trend indicates that new microalgae growth is entering an exponential phase. The highest growth rate was achieved by the *Chlorella:Spirulina* consortium at a 3:2 ratio of 0.021/hour at the 110th hour, then the 2:1 ratio consortium was 0.016/hour at the 130th hour, then the 1:1 ratio consortium was 0.013/hour. hours at 130 hours, then *Chlorella vulgaris* at 0.012/hour at 130 hours, and *Spirulina platensis* at 0.007/hour at 200 hours.

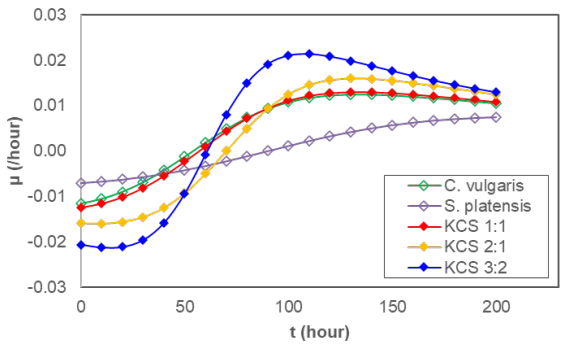

Fig. 3: Specific Growth Rate Curve of Microalgae and Consortium

To support the application of microalgae in MmFC which relies on oxygen levels in the liquid at the electrodes, measurements of dissolved oxygen levels were carried out during the growth of microalgae. In Figure 4, it was observed that dissolved oxygen levels continued to increase during the cultivation period along with an increase in the concentration of microalgae cells in culture. This shows that at higher concentrations of microalgae, higher oxygen can be produced, so that high concentrations of microalgae in culture have the potential to improve the performance of Microalgae-microbial Fuel Cell (MmFC).

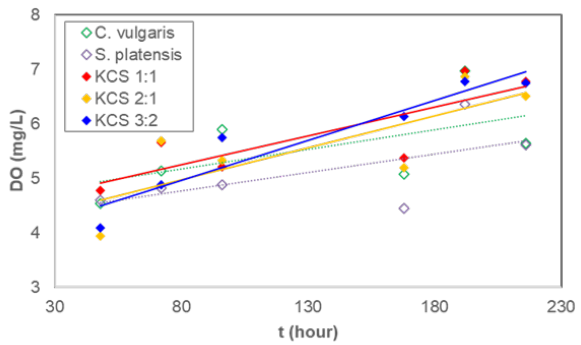

Fig. 4: Dissolved Oxygen Content in Microalgae and Consortium Culture

The oxygen produced by microalgae is related to its photoautotrophic properties which allow microalgae to carry out photosynthesis. The process of photosynthesis is the process of assimilation of carbon dioxide with photon energy from light absorbed by chloroplasts into essential organic compounds in the process of growth and regeneration[21].

Based on the results obtained, it was found that the *Chlorella: Spirulina* consortium at a ratio of 3:2 had the highest growth rate of up to 0.021/hour and produced the highest biomass of 3.94 g/L followed by other consorstium ratio. Thus, in the next stage the consortium of microalgae will be used in MmFC.

3.2 Microalgae Consortium Growth in MmFC Reactor

In MmFC, *Chlorella:Spirulina* culture was more green-concentrated on the second day compared to the first, but became yellowish on the third. Visually, this shows an increase and decrease in biomass on the second and third days. Recorded optical density of microalgae culture supports the visual appearance as MmFC operates.

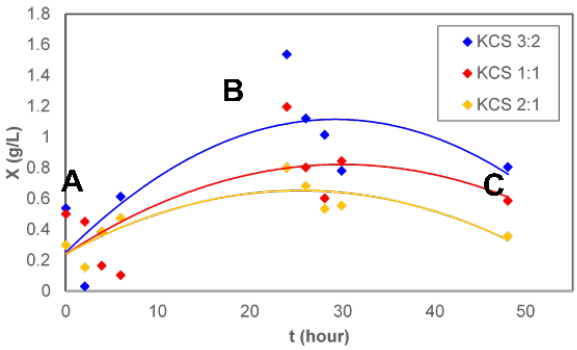

Fig. 5: Biomass Growth Curve of Microalgae Consortium in MmFC Reactor

Figure 5 shows that the density of microalgae cells tends to increase on the second day but decreases on the third day. The decrease in cell density can be caused by conditions that are not under the needs of microalgae thus entered the stationary growth phase, such as a lack of carbon dioxide levels. In this study, the gas from the anode compartment was not connected to the cathode compartment so that the carbon dioxide source for microalgae only relied on the binding of carbon dioxide from the atmosphere (0.03% CO_2)[27] which was originally intended to produce added value in MmFC with microalgae as a carbon capture agent. Besides, the microalgae have also passed the preculture stage, so it is possible for the microalgae to be in the stationary phase. However, in this case, further research is needed to identify the specific cause. The highest cell density was achieved by the *Chlorella:Spirulina* consortium 3:2 ratio at 1.537 g/L.

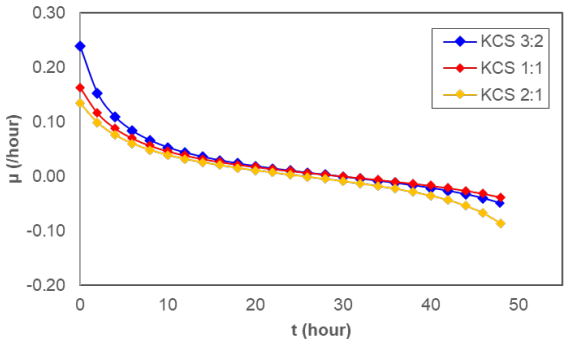

Fig. 6: Specific Growth Rate Curve of Microalgae Consortium in MmFC Reactor

Figure 6 shows a trend that shows a high growth rate at the beginning of the cultivation period which illustrates that growth is in an exponential phase where the highest growth rate is 0.24/hour. But as the cultivation period in MmFC, the longer the growth rate decreases to zero at the peak of the growth curve (reaching the highest concentration). When the growth curve decreases, the microalgae growth rate is negative. These results support the statement that the use of microalgae in energy sector is very promising but the way how to use microalgae potential efficiently and develop appropriate technology still become the biggest challenge[28].

3.3 Bioelectricity Production from MmFC Reactor

The level of electrical energy generated in MmFC is influenced by the type of microbe and substrate as well as the type of microalgae and its medium which affects the potential level of reduction and oxidation in the fuel cell. In addition, the type of membrane and electrode also affects because the type of membrane is related to the transfer of protons from the anode to the cathode compartment, while the electrode is related to the transfer of electrons from the anode to the cathode compartment. When the reduction potential is large, the ability to bind electrons is greater. Conversely, when the oxidation potential is large, the ability to lose electrons will be greater. This affects the number of electrons that pass through the external circuit and produces a reverse current or current flow from the cathode (+) to the anode (-) which

is commonly referred to as electric current.

Figure 7 shows that on the first day the energy increased sharply, on the second day the energy increased slightly, then on the third day, it tended to be constant. The highest voltage achieved was 0.4371 V with a power density of 323.477 mW/m^2 at 24 hours. Based on the electrical voltage data obtained and the resistance used, it can also be determined the electric current generated by MmFC. In this study, the highest current achieved by the *Chlorella: Spirulina* consortium was 4.371 mA at 24 hours.

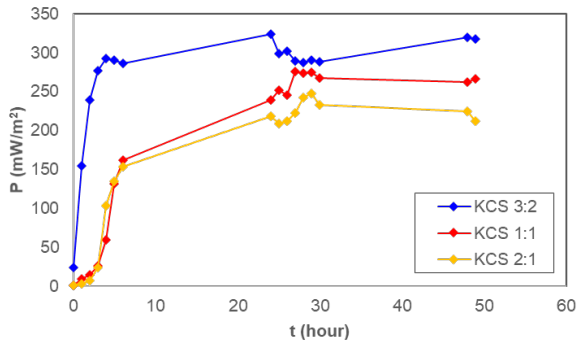

Fig. 7: MmFC Power Density Curve

In the research of Utami et al. (2018)[13] which used tempe waste in MFC, obtained an electric voltage of 291.1 mV and electrical energy of 66.33 mW/m^2 so that the MmFC design in this study gave results with a higher electrical potential. This result can be attributed to the use of microalgae in the cathode compartment which provides more efficient oxygen for the reduction reaction. In addition, the dual-chamber configuration used in this study supports the different conditions at the cathode and anode which causes a high potential difference in the MmFC compared to the use of a single chamber.

In MmFC study by Rosyadi et al. (2017)[15] using tempe wastewater and *Cladophora* macroalgae, obtained the electrical voltage that is close to the results in this study, 320 mV or 0.320 V. It is because the research uses the same type of waste as this study and uses photosynthetic algae but with different species. This shows that the type of waste used in the anode compartment has a significant effect on the potential difference of MmFC as a source of electrons in the MmFC circuit. In another study, by Huarachi-Olivera et al. (2018)[14], obtained electrical energy which is slightly higher than the maximum gain in this study, which is 327.67 mW/m^2. This can be caused by a larger working volume compared to this study, so it has a greater capacity to carry out redox reactions. In addition, the concentration of microalgae biomass is much higher so that it can produce more oxygen in the cathode compartment. Table 1 shows the comparison between MFC components and bioelectricity production in a number of studies that have been carried out.

Table 2. Comparison with Other MmFC Research

Ref	Bioelectricity Produced	Waste	Algae
14)	954 mV; 327.67 mW/m^2	Industry	*C. vulgaris*
29)	166.5 V; 44.33 mW/m^2	Tapioca	*S. platensis*
15)	320 mV; 1293.15 µW/m^2	Tempe	*Cladophora*
30)	0.115 V	Batik	*C. vulgaris*
31)	171.44 mW/m^2	Tofu	*S. platensis*
This Paper	0.4371 V; 323.48 mW/m^2	Tempe	*C:S*

Regarding its application to produce bioelectricity, the value obtained from MmFC in this study is relatively low. Small led lights require an electric current of 10 mA to 20 mA at a voltage of 1.6 V to 3.5 V depending on the character of the color produced. Therefore, the MmFC electricity gain is still not able to turn on the LED lamp. It is necessary to increase the result several times from the results obtained in this study by optimizing the growth of microalgae in the cathode compartment or increasing the surface area of the electrodes and membranes.

4. Conclusion

In the preliminary procedure of microalgae cultivation (before MmFC operation), the dry weight of the microalgae consortium biomass was higher than that of the pure microalgae biomass where the dry weight of the microalgae consortium *Chlorella:Spirulina* with a ratio of 3:2 was 3.94 mg/L and the maximum growth rate was 0.021/hour. ; 1:1 ratio consortium of 3.86 mg/L and 0.013/hour; a 2:1 ratio consortium of 2.01 mg/L and 0.016/hour; *Chlorella vulgaris* ie 0.76 mg/L and 0.012/hour; and the lowest was *Spirulina platensis*, at 0.55 mg/L and 0.007/hour. These results indicate that the microalgae consortium *Chlorella vulgaris* and *Spirulina platensis* has the potential to improve the performance of MmFCs compared to using pure microalgae cultures.

In the operation of the MmFC device, a consortium of microalgae *Chlorella vulgaris* and *Spirulina platensis* at a ratio of 3:2 resulted in dry weight biomass and maximum growth rates at 1.537 g/L and 0.24/hour with dissolved oxygen levels reaching 5.3-6.3 mg/hour L. This consortium also produces an electrical voltage of 0.4371 V and electrical energy (power density) of 323.477 mW/m^2 using a 100-ohm resistor and an anode surface area of 59.06 cm^2.

Acknowledgements

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Nomenclature

KCS *Chlorella:Spirulina* consortium

OD540	optical density at 540 nm
OD486	optical density at 486 nm
X	dry weight of cells (g/dm^3)
μ	specific growth rate (hour^{-1}),
t	time (hour)
P	power density (mW/m^2)
V	electric voltage (V)
I	electric current (A)
A	anode surface area (m^2)
R	resistance (ohm)
E^0_{red}	standard reduction potential (V)

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