

Production of Bio-Electricity from Sidoarjo Mud and Molasses Using Microbial Fuel Cells (MFCs) Assisted External Resistance Technology

Raden Darmawan^{1,*}, Sri Rachmania Juliastuti¹, Nuniek Hendriani¹,
Veby Saragih¹, Kelita Mea Melaca¹, Sandra Sopian¹ and Masato Tominaga^{2,3}

¹Department of Chemical Engineering, Faculty of Industrial Technology and Systems Engineering, Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia

²Department of Chemistry and Applied Chemistry, Saga University, Saga 8408502, Japan

³Graduate School of Science and Engineering, Saga University, Saga 8408502, Japan

(*Corresponding author's e-mail: rdarmawan@chem-eng.its.ac.id)

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Abstract

Mud contains little organic matter but has the potential for microbial electricigens (electrogenic) (4.5 - 6.6 mV) this is because the mud comes from bursts from below the earth's surface containing magma and hydrothermal fluids. Therefore, this study aims to learn the addition of Sidoarjo mud (a source of microbes as a biocatalyst) and molasses into microbial fuel cells technology for external resistance variations in generating bioelectricity. Referring to this goal, variations in molasses (2.5, 5.0 and 10 % w/w) and external resistance (1, 10, 100, 1,000 and 5,100 k Ω) were carried out. The stages in this study include starter preparation by assembling MFCs using carbon electrodes connected to electronic devices. Furthermore, the mixture of mud and molasses was put into the MFCs by immersing the anode while the cathode was on top of the mud. After that, measuring the electricity potential (voltage and power density) on various external resistances and molasses. The optimum results for the electric voltage and the best power density were obtained when using an external resistance of 1 k Ω and adding 2.5 % molasses with, respectively 210.25 mV/m² and 38.91 mW/m² (electric current 51.75 mA/m²). Based on the results of the SEM-EDX analysis for carbon electrodes, it was found that there was a change in composition from before the process to 9 weeks of running time, where the previous carbon content was 97.27 to 0.67 %. In addition, there were other contents such as sulfur, silica, ferrum, phosphorus and other elements caused by bacterial metabolism in biochemical degradation of organic components.

Keywords: External resistance, Molasses, Bio-electricity, Sidoarjo mud, MFCs

Introduction

During 2019, global energy needs were supplied by at least almost 84 % from non-renewable sources such as oil, natural gas and coal. While 64 % of the electricity generated comes from the process of burning fossil fuels [1]. In fact, as is known, the burning of fossil fuels is not only a major factor in global warming, but its exploitation which can continuously disrupt many ecosystems [2]. Whereas, there are many requests to limit the use of these fuels [3]. The implication is that research related to new and renewable energy has increased, especially in the categories of biomass, solar and wind energy [4]. Even a search for the keyword "renewable energy" on the *sciencedirect* site shows almost 175,000 in the last 5 years (2017 - 2021). Among the many keywords, the Microbial Fuel Cells (MFCs) system, which is a set of renewable energy technologies that often appears, reaches 13 % of the total keywords.

MFCs were first developed by M. C. Potter in 1911. This biotechnology is a device consisting of an anode electrode, a cathode and an electronic device to convert chemical energy into electrical energy by using bacteria as a catalyst to oxidize organic and inorganic materials [5,6]. Microbes commonly used in MFCs are bacteria electricigens (electrogenic) such as *Shewanella putrefaciens*, *Geobacteraceae sulfurreducens*, *Geobacter metallireducens* and *Rhodospirillum rubrum* [7]. This species was chosen because it is capable of producing a metabolism that can break down organic matter into hydrogen (H₂) and oxygen (O₂). Hydrogen is the raw material used for reduction reactions with oxygen, thereby releasing electrons at the anode as a source of electric current.

On the other hand, the Sidoarjo mud is a hot mudflow event at the PT. Sidoarjo Brantas Sidoarjo, East Java on May 29, 2006 [8]. Sidoarjo mud in Sidoarjo is composed of 62.14 - 73.29 % water and the rest is solid [9]. The salinity of the mud is very high (1.71 - 2.43 mS/cm), pH value is around 6.8 - 7, exchange

capacity cations of 11.42 - 32.57 (Me/100g), and redox potentials of 4.5 - 6.6 mV [10]. Mud contains little organic matter but has the potential for microbial electricigens (electrogenic) this is because the mud comes from bursts from below the earth's surface containing magma and hydrothermal fluids [11]. Seeing the potential as an alternative source of electricity in the future, the utilization of Sidoarjo mud using MFCs can be considered as a promising technology to generate electricity (bioelectricity).

In supplying organic matter (substrate) in MFCs, various previous studies have utilized glucose [12], fatty acids [13], acetate [14]. However, in reviewing effective and efficient research to support environmentally sustainable development programs, molasses waste becomes a promising carbon source. This is because the waste from the sugar factory still contains reducing sugars up to 48 - 56 % [15]. Reducing sugar is a compound that is easily digested and can be directly absorbed by the blood for metabolic processes to obtain energy. The addition of molasses to the sludge can be useful as an alternative organic source for the microorganisms contained in the sludge as well as the utilization of waste from sugar factories. In addition, this statement is confirmed by a study conducted by Arungovind *et al.* [16] that substrates from various sources such as livestock waste, molasses, rice washing water and industrial wastewater, individually and also in combination can be used in MFC cells to generate electricity. With the results of the electric voltage in a mixture of rice washing water and molasses of 222 mV at 60 h of operation [16].

However, in the application of MFCs, the use of external resistance based on the numerical model presented by Picioareanu *et al.* [17] is able to produce (1) higher microbial growth and (2) the current generated at the anode can be reduced [17]. Thus, Katuri *et al.* [18] in his study stated that differences in external resistance can cause several significant differences in the operation of MFCs, including the external resistance used, 0.1 k Ω is able to produce the largest current, which is 273.61 mA/m² [18].

Therefore, seeing the potential of the various studies described previously, this study aims to review the utilization of Sidoarjo mud and molasses in producing bioelectricity using Microbial Fuel Cells (MFCs) assisted by variations in external resistance. The electricity generated in these MFCs can be used as an alternative substitute for fossil energy so as to limit the use of these fuel sources.

Materials and methods

Materials

The materials used in this study were Ti-wire, copper, carbon cloth (Carbon felt GF-20, Nippon Carbon Ltd), NaOH (Merck), (NH₄)₂SO₄ (Merck), aquadest, Na₂HPO₄ (Merck), MgSO₄·7H₂O (Merck), NH₄NO₃ (Merck), KH₂PO₄·3H₂O (Merck), K₂HPO₄ (Merck), FeCl₃·6H₂O (Merck), CaCl₂ (Merck), yeast, FeCl₃·6H₂O (Merck), CaCl₂ (Merck). The *molasses* to be added to Sidoarjo mud were obtained from the nearest sugar factory in East Java - Indonesia (**Table 1**).

Table 1 Physicochemical of Sidoarjo mud [19].

No.	Characteristic	Composition	Unit
1	Physical characteristic		
	Density	1.30 - 2.34	g/cm ³
	Porosity	44.50 - 46.75	%
	Sand	8.00 - 20.00	%
	Ash	39.00 - 46.00	%
2	Chemical characteristic		
	pH	6.60 - 7.00	-
	Pb (lead)	0.27 - 0.34	mg/kg
	Cu (copper)	0.83 - 1.31	mg/kg
	Water content	40.41 - 60.73	%
	C-organic content	54.75 - 55.47	%

Microbial fuel cells instrument

The device of this study used the single-chamber reactor. The instrument design according to the Figure 1.

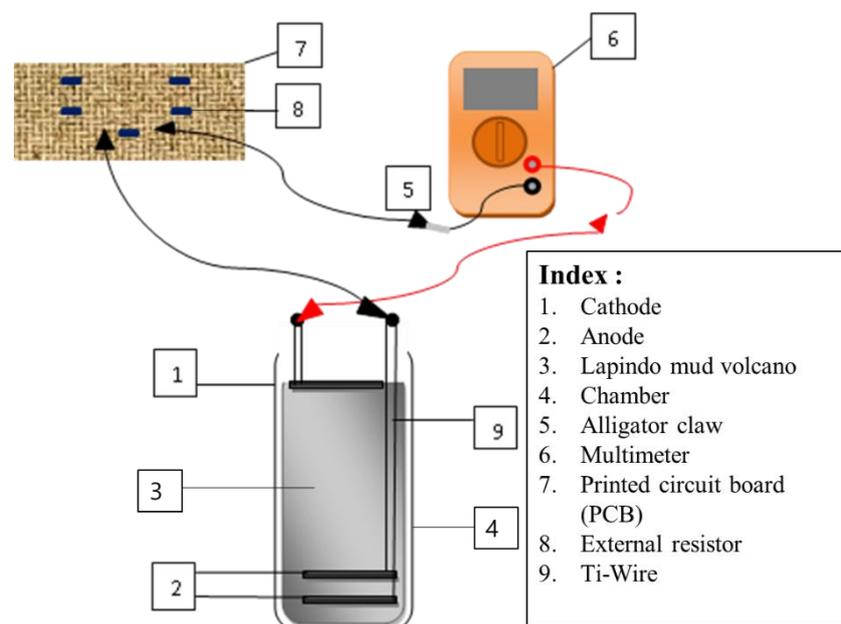


Figure 1 Microbial fuel cells assisted external resistance instrument.

Experimental design

The operation condition of this study were 1 kg of mud total mass, room temperature (30 °C), pressure of 1 atm, pH of 7 - 8, height of anode and cathode, 3 and 5 cm, respectively and size of electrode, 2×5 cm². While the variable of this study were molasses (2.5, 5 and 10 % of Sidoarjo mud mass) and external resistance (1, 10, 100, 1,000 and 5,100 kΩ).

Optimization using response surface methodology

Optimization in this study was carried out using the Response Surface Methodology (RSM) with type I-optimal. Quadratic modeling and optimization was performed using design expert software version 13.0.9.0 (State-ease Inc., Minneapolis, MN, USA).

Table 2 Factor parameters in experimental design.

Factor	Name	Units	Type	Minimum	Maximum	Mean	Std. Dev.
A	Concentration of molasses	%	Numeric	2.50	10.00	6.16	3.08
B	Time	Week	Numeric	0	9.00	4.39	3.21
C	External resistance	Ohm	Categoric	1	5,100	Levels:	5.00

In these factor parameters, the responses determined in this study are voltage (mV/m²) and power density (mW/m²).

Starter preparation

Carbon cloth was cut in size 2×5 cm² and then dipped into a 0.3 M NaCl solution in order to remove air bubbles inside the carbon cloth. Mud and molasses were weighed according to the variables. The 1 N NaOH solution was added to the molasses until the pH is about 7. The carbon electrode and Ti-wire cable were combined with weaving. In chamber mounted 2 anodes and 2 cathodes. Both anodes were mounted on a chamber with a height of 3 cm and 5 cm from the bottom of the chamber. Both cathodes were mounted to an altitude where the carbon cloth touches the surface of the mud. While the dominant type of bacteria added to the external the best resistor and medium with a sum of 10¹¹ - 10¹² cell/mL were *Shewanella oneidensis MR-1* and *Escherichia coli*.

The measuring of electricity potential

Electrical voltage measurements and electric current were performed using a multimeter tool equipped with a PCB tool circuit. The PCB consists of an external circuit of resistance that is 1, 10, 100, 1,000 and 5,100 k Ω . External resistance was connected to the multimeter and cathode/anode devices with connectors (alligator claws). The voltage and the electric current values shown on the multimeter and then recorded.

The bacteria amount analysis

In the analysis of bacteria amount, samples were taken and weighed as much as 1 g. Then the sample was diluted in the test tube with the addition of aquadest up to 10 mL then dilution is performed according to the desired dilution level. The sample was taken one drop by using a dropper on the surface of the *hemocytometer*. Then the *hemocytometer* was placed under the microscope lens to calculate the number of cells. Observations were made using a microscope with 400 \times magnification.

Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) analysis

The sample to be tested was a piece or side of a carbon cloth electrode with variable 5 % molasses with Ti-Wire 9th week. Before scanning with SEM and EDX photo machines, the sample goes through several steps (1) Preparing the sample to be tested, (2) Cutting the sample to size on the SEM photo machine (3) Sanding the sample to be tested (4) Coating the surface of the sample to be tested (6) Laying the sample to be tested ready to be tested into the SEM photo machine and (7) Scan or process SEM and EDX.

Results and discussion

Microbial growth

Growth curve is an information about the life phase of a bacteria used to determine the rate of cell growth and the influence of the environment on the rate of growth. This curve consists of 4 main phases, namely lag phase, exponential growth phase, stationary phase and phase of population decline. Preparation of bacterial growth curve is an important part in a study because it describes bacterial colonization. Time generation calculations are needed to determine the population prediction of each microorganism in the same time period as its activity in metabolic processes. Steps taken to determine the growth curve of bacteria is by bacterial isolation.

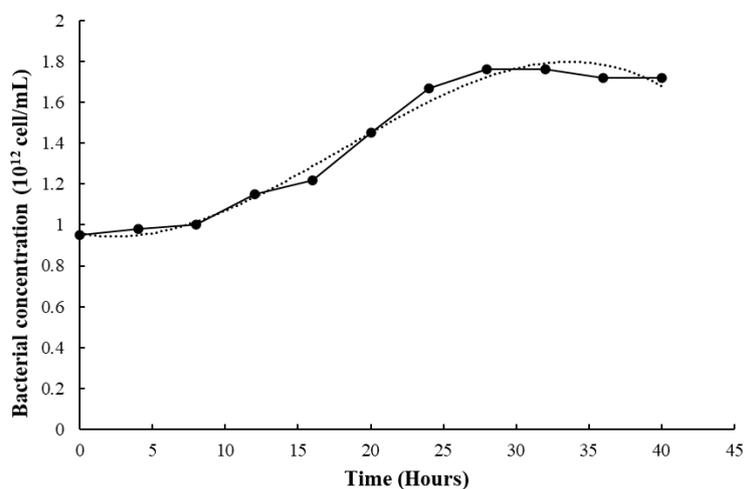


Figure 2 The growth of *Shewanella oneidensis MR-1* bacteria.

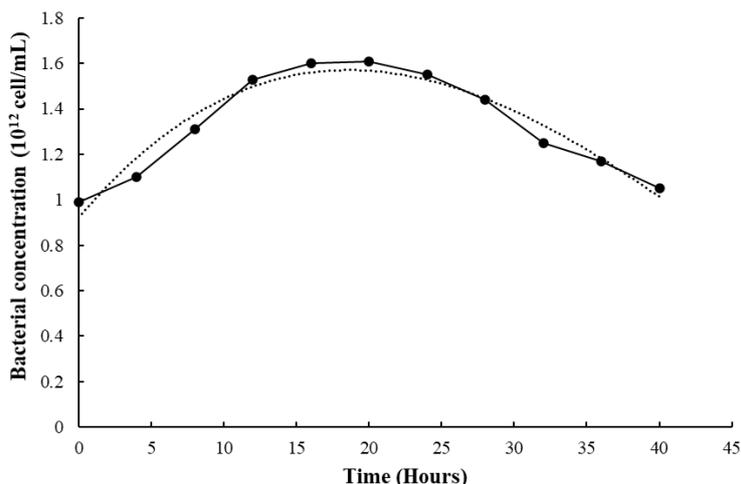
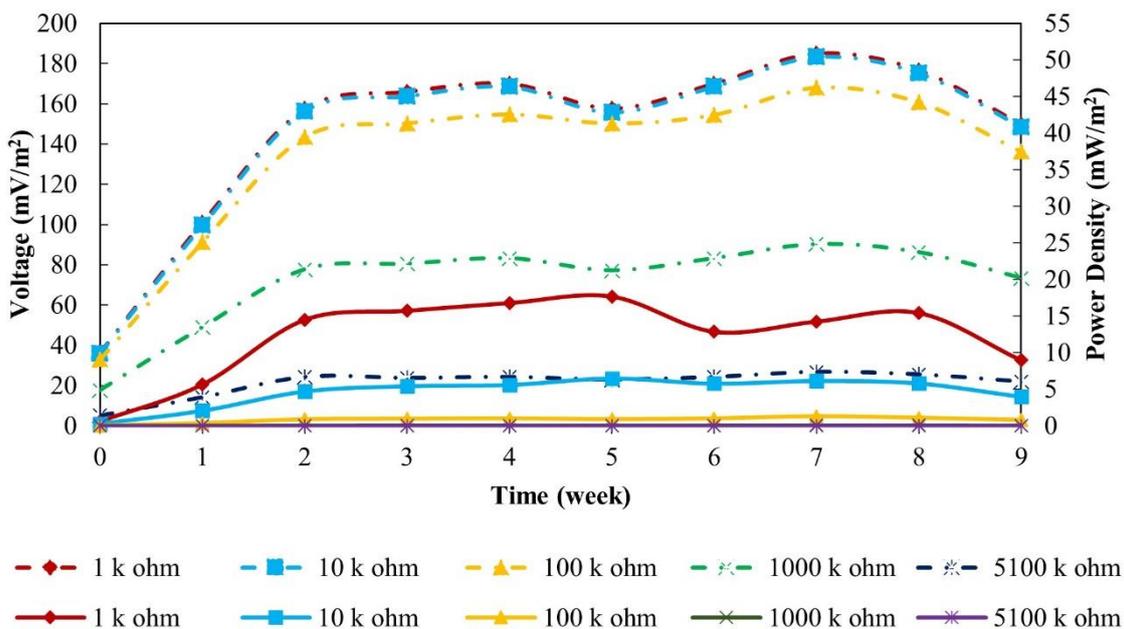


Figure 3 The growth of *Escherichia coli* bacteria.

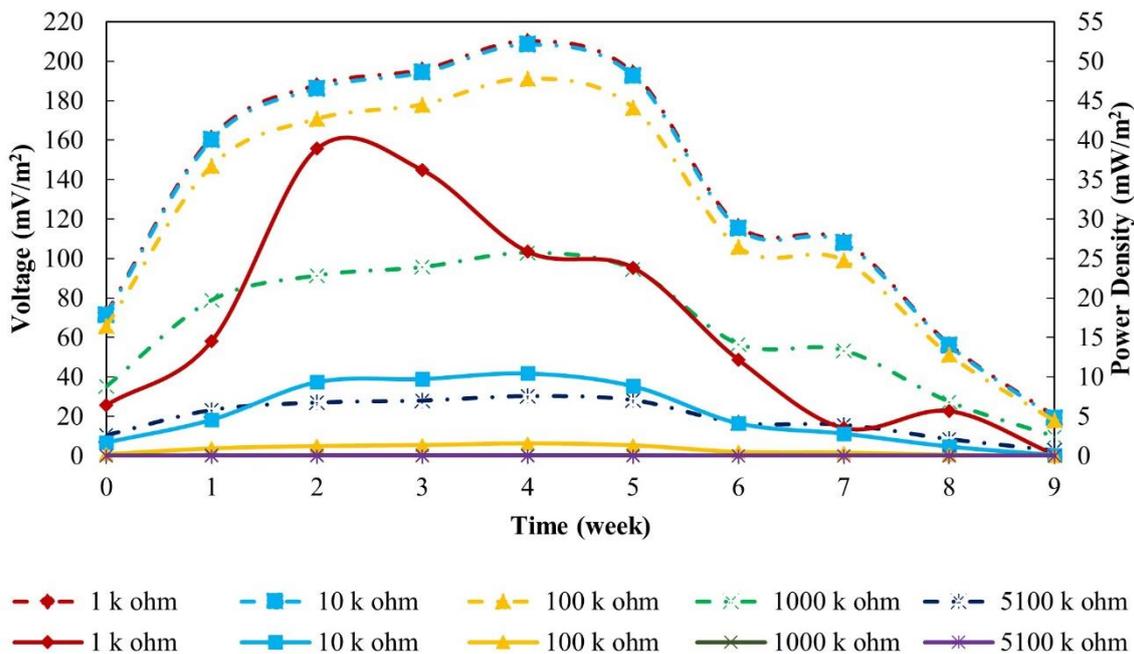
When the active bacteria metabolize, it is expected that there will be many electrons and protons generated so that the biolocyte increases. Thus, the 2 predominant bacteria were introduced into the reactor after 16 h of incubation for the bacteria *Shewanella oneidensis MR-1* and after 8 h of incubation for the dominant addition of *Escherichia coli* bacteria where the log phase was occurring.

The effect of external resistance

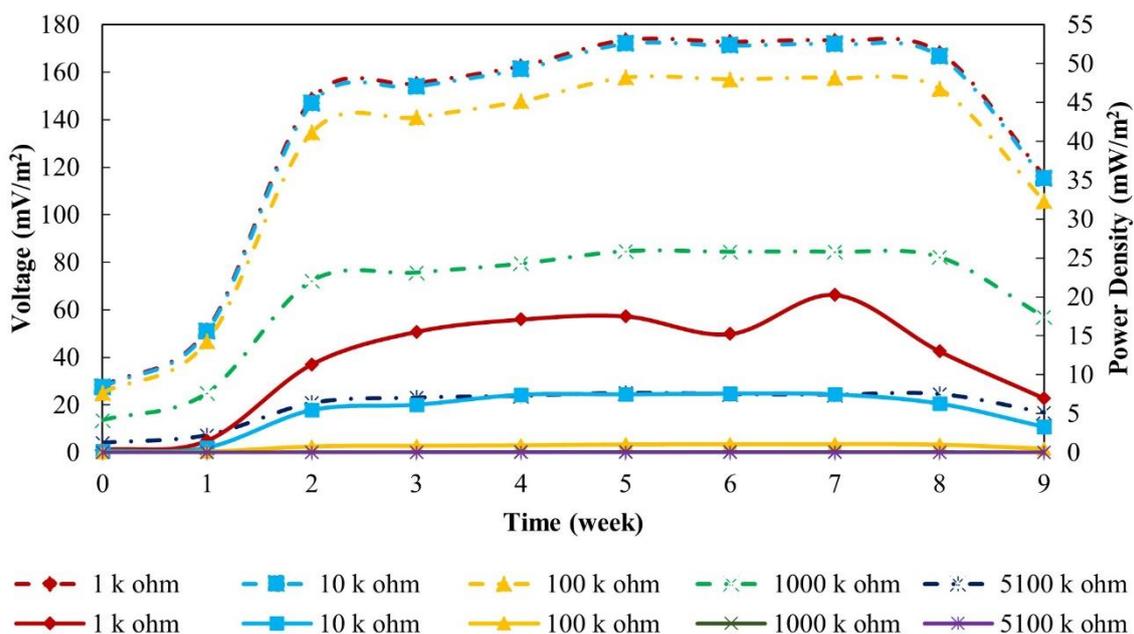
External resistance directly affects potential and electric current, and also affects other variables within MFCs, such as bacterial diversity, biofilm morphology, power generated, coulombic efficiency and stability. Therefore, the optimal selection of external resistance to obtain good performance in the MFCs system is essential. As a consequence of the high external resistance, the diversity of the microorganism changes and the power will be low. This can be related to the internal resistance which is the obstacle that arises because it is influenced by density, wire length and wire cross section. In this research was conducted by using 5 external variables of resistance that was 1, 10, 100, 1,000 and 5,100 kΩ.



(a)



(b)



(c)

Figure 4 The comparison of voltage (full line) and power density (dotted line) with time by concern of various external resistance and addition (a) molasses 2.5 %, (b) molasses 5 % and (c) molasses 10 %.

Based on **Figure 4** for each case (a), (b) and (c) shows the comparison of voltage and power density every week on various external resistance variations. The picture above shows the fluctuating conditions in the electric voltage and the resulting power density. However, for each **Figure 4(a) - 4(c)** the highest voltage is achieved when using an external resistance of 1 kΩ which is 210.25 mV/m² each (at week 4); 185 mV/m²

(at the 7th week) and 173.5 mV/m² (at the 5th week). Similar results are also shown in power density which gets the highest gain for each **Figure 4(a) - 4(c)** at 1 kΩ external resistance with each of 38.916 mW/m² (in the 2nd week); 17.668 mW/m² (at week 5) and 20.27 mW/m² (at week 7).

External resistance directly affects electric potential and current, and also affects other variables in MFCs, such as bacterial diversity, biofilm morphology, yield power, coulombic efficiency and stability. Therefore, the selection of the optimal external resistance to obtain good performance in the MFCs system is very important. The external selection of resistance can select the growth of the microbial population in the anode chamber of the MFCs system. As a consequence of the high external resistance, the diversity of microorganisms changes and the power will be low. This can be related to internal resistance which is the resistance that arises because it is influenced by density, wire length and wire cross-sectional area. So we get Ohm's law:

$$I = \frac{V}{R_{ext} + R_{int}} \tag{1}$$

$$P = I^2 \times R_{ext} = \frac{V}{(R_{ext} + R_{int})^2} \times R_{ext} \tag{2}$$

$$P = \frac{V}{(R_{ext} + R_{int})^2} \times R_{ext} \tag{3}$$

The maximum power transfer theorem states that the maximum power is obtained when the external resistance is equal to the internal resistance, i.e. when $(R_{int})^2 = (R_{ext})^2$. If the external resistance is greater or less than the internal resistance, the power will drop [20]. This is in accordance with the results obtained in this study, namely in **Figure 4**. The results of a similar study were also shown by the study of Katuri *et al.* [18] who obtained the highest power density on the external resistance variable 1 kΩ.

The effect of molasses

The effect of adding *molasses* to the generated electricity is shown in **Figure 4**. This study was conducted by using five variables of addition of *molasses* that are 2.5, 5 and 10 %. Measurements were made for 9 weeks.

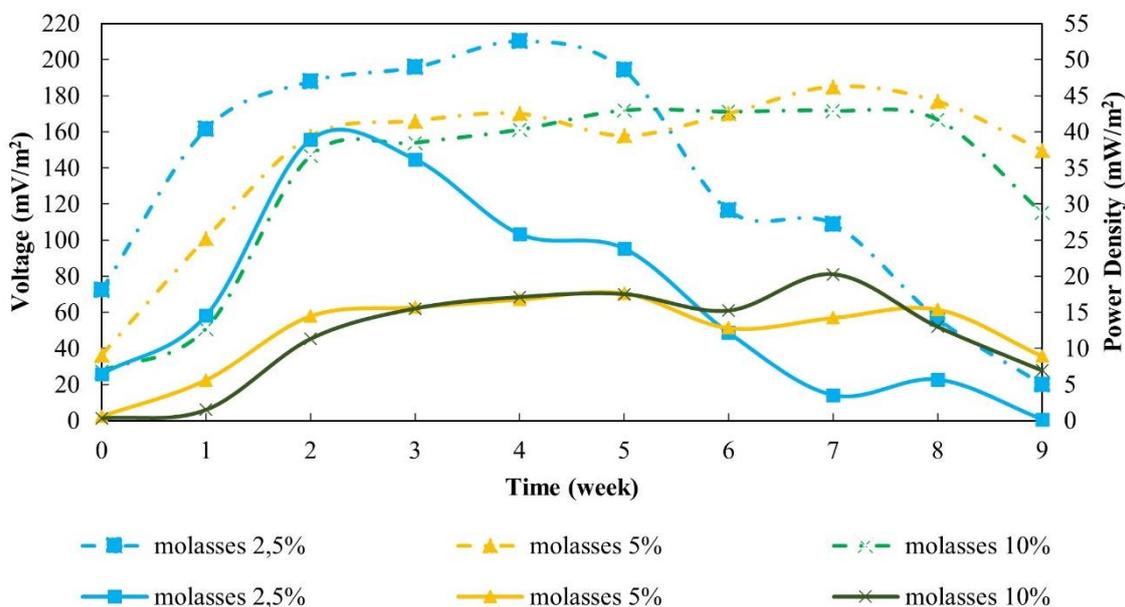


Figure 5 Comparison between power voltage (full line) and power density (dotted line) with time for variable addition of molasses using external resistance 1 kΩ.

Figure 5 shows the most optimum medium, namely Sidoarjo mud with the addition of 2.5 % molasses where the electric potential generated at week 4 is 210.25 mV/m² using an external resistance of 1 kΩ. In

the picture there is an increase in electricity voltage until the 4th week and then it decreases in the 5th week. Furthermore, the value of the electric voltage tends to be constant from the 6th week to the 7th week and shows a gradual decline again. In contrast to the power density, the gain was 36.214 mW/m² in the 2nd week, where the increase in electrical voltage occurred until the 2nd week and then decreased in the 3rd week. Furthermore, the value of the electric voltage tends to be constant from the 3rd week to the 5th week and shows a gradual decline again.

Molasses as a by-product of the cane sugar production process, has been widely used to produce renewable energy such as ethanol and biogas. The sucrose contained in molasses is used as the main carbon source for MFCs. Substrate molecules spread through the biofilm, which are deposited on the surface of the anode, and are oxidized by bacteria residing in the film. A higher COD level of molasses results in a higher stress, as more sucrose molecules are oxidized. A study conducted by Sirinutsomboon [21] stated that there was an effect of differences in biofilm thickness on stresses with higher COD levels. If the biofilm is too thin, the small number of bacteria cannot keep up with the high amount of sucrose. The electricity generated does not increase as the amount of sucrose increases [21]. In this study, the best electric potential was achieved when using Sidoarjo mud with the addition of 2.5 % w/w molasses. This happens, it is possible that the combination of Sidoarjo mud (97.5 % w/w) which contains a number of electricigen bacteria with a carbon source from molasses is the optimum medium.

The effect of addition dominant bacteria

In this study, the addition of dominant *Shewanella sp MR-1* and *Eschericia coli* (with ratio ratio 1:1) to reactor when log phase occurred. The addition of the dominant bacteria is intended to find out how the effect on the electrical potential generated compared to only utilizing bacteria derived from mud.

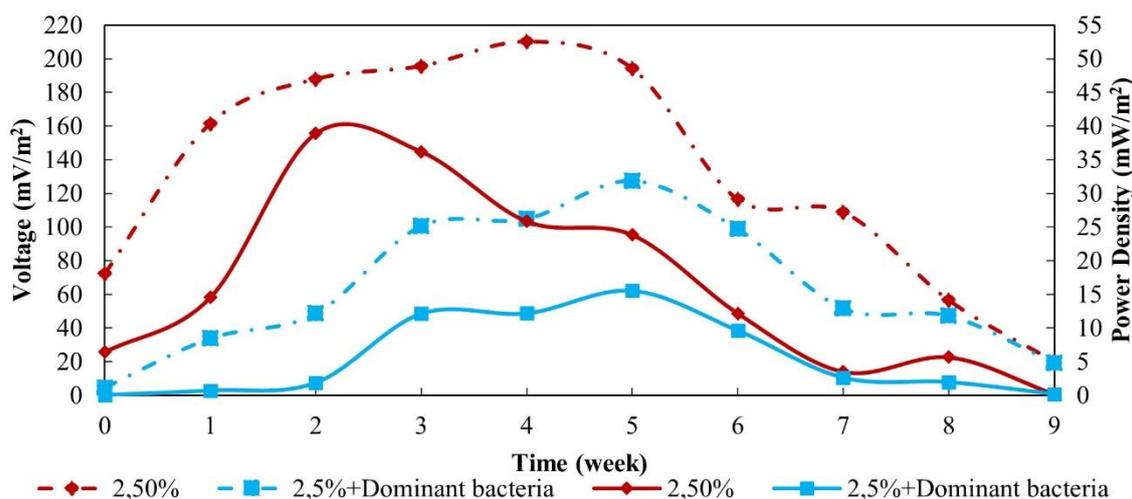


Figure 6 Comparison between power voltage and power density with time for addition of molasses 2.5 % using external resistance 1 kΩ.

For 2.5 % molasses variables without bacterial dominant addition, the highest voltage reached 201.25 mV/m² at week 4 and the highest power density value reached 38.91 mW/m². As for the molasses 2.5 % variable with the addition of the dominant bacteria achieved the power voltage of 127.43 mV/m² and for the highest power density of 15.51 mW/m². From the figure, it is shown that the 2.5 % molasses variable with the dominant addition of bacteria can result in lower voltage and power density compared to 2.5 % variable without the addition of dominant bacteria. This is because the activity and population of more microorganisms with the number of substrate that is increasingly decreasing day because the volume of reactor still causes the seizure of food among bacteria cells.

The test result of SEM-EDX

Scanning Electron Microscopy (SEM) is an electron microscope used to observe surface morphology in micro and nano scales. The EDX or *Energy Dispersive X-ray* test is used to identify the composition of the elements contained in the sample.

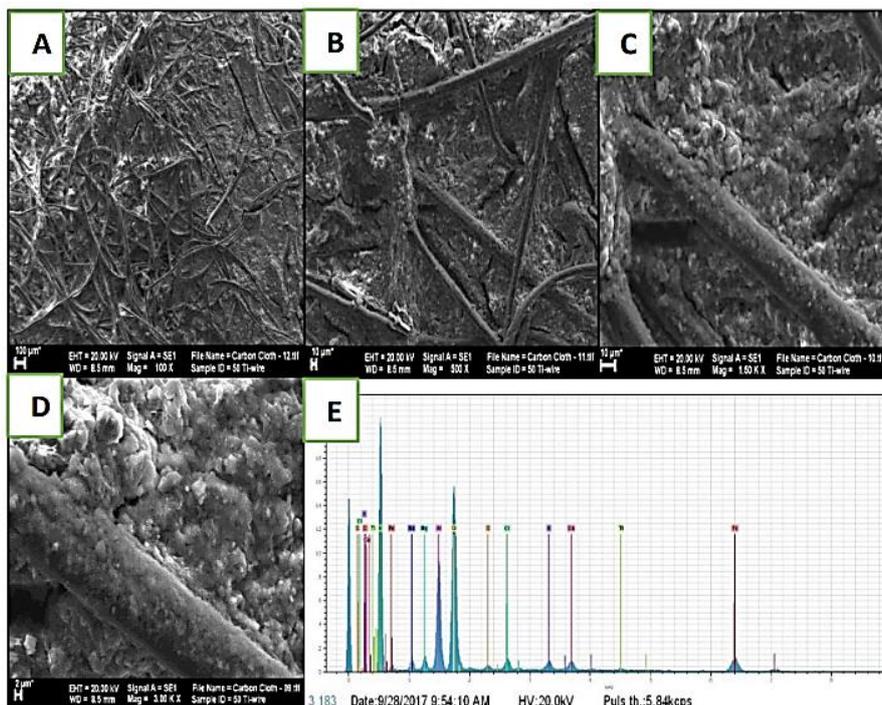


Figure 7 Analysis of SEM- EDX carbon cloth electrode with 5 % molasses with Ti-Wire 9th week.

The result of SEM-EDX analysis of *carbon cloth* electrode with variable of 5 % *molasses* by using Ti-wire after 9 weeks experiment in the laboratory is shown in **Figure 9** where A, B, C and D showed SEM results with magnification 100×, 500×, 1,500× and 3,000×, while E shows the result of EDX spectrum at 3,000× magnification. The SEM results show a biofilm formed on the anode surface. The biofilm formation is seen from the uneven surface morphological structure of the carbon cloth from the initial conditions (Magnification 3,000×). Over time, the biofilm will grow and become thicker so as to cover the anode. This causes the substrate to not reach the inner bacteria of the biofilm resulting in decreased performance of the cells.

Table 3 The results of the SEM-EDX test on carbon cloth with Ti-wire on the addition of 5 % molasses at week 0 (t_0) and week 9 (t_9).

Carbon Cloth t_0		Carbon cloth t_9	
Element	Composition (% weight)	Element	Composition (% weight)
C	97.27	C	0.67
O	2.73	O	61.15
		Si	15.35
		Al	9.83
		Fe	6.39
		Ca	1.44
		Na	1.37
		K	1.07
		Cl	1.04
		Mg	1.03
		Ti	0.40
		S	0.26
Total	100	Total	100

Based on **Table 3**, it can be compared the results of the pure carbon cloth test with the element C content (97.27 %) undergoing degradation after 9 weeks of experiments carried out in the laboratory with a decrease in the C content to 0.67 %. When a carbon cloth is used, the carbon cloth will experience a

decrease in the carbon composition of the anode so that after a certain time the performance of the MFCs will begin to decline. The appearance of elements other than C at t_9 is the result of bacterial metabolic activity, in this case the biochemical degradation of organic components [22]. **Table 1** shows the degradation of organic components (C) and the formation of new components after MFCs operate. The EDX spectrum also shows damage to the components of the carbon cloth at the anode as shown in **Figure 8**. In long-term operation, after a certain time the performance of MFCs begins to decrease. In addition, the presence of dead bacteria on the inner or outer surface of the biofilm prevents the substrate from reaching the bacteria in the inner layer. It causes reduced cell performance.

Optimization of bio-electricity from Sidoarjo mud and molasses using MFCs

The factor used in optimizing the results obtained (bio-electricity) is the molasses concentration factor by setting 3 levels (2.5, 5 and 10 %), time (0 - 9 weeks with a data collection range of 1 week) and external resistance (1, 10, 100, 1,000 and 5,100 k Ω). Based on the design type used is i-optimal (custom) on the response surface methodology, then 28 runs are given with a quadratic model design.

Table 4 Result of data design (actual) optimization using response surface methodology with type i-optimal.

Run	Factor A A: Concentration of molasses (%)	Factor B B: Time (weeks)	Factor C C: External resistance (k Ω)	Response 1 Voltage (mV/m ²)	Response 2 Power density (mW/m ²)
1	5	0	1,000	17.70	0.0018
2	2.5	6	5,100	16.75	0.0020
3	10	0	1	27.70	0.3601
4	5	5	1,000	77.30	0.0464
5	10	7	1,000	84.30	0.0592
6	10	1	1,000	25.00	0.0050
7	5	9	100	136.30	0.7903
8	5	5	10	155.80	0.0416
9	2.5	4	1	210.25	25.8608
10	2.5	4	100	191.25	1.5683
11	10	2	5,100	0,00	0.0021
12	5	9	5,100	22.00	0.0022
13	5	8	1	176.80	15.4126
14	10	0	10	27.40	0.1699
15	2.5	1	5,100	23.25	0.0020
16	5	1	1	101.00	5.6358
17	5	0	100	33.00	0.0461
18	10	5	100	157.80	1.0254
19	2.5	0	10	71.50	1.6731
20	2.5	9	10	19.20	0.0787
21	10	9	10	115.30	3.2846
22	10	2	5,100	0.00	0.0021
23	5	8	1	176.80	15.4126
24	10	5	100	157.80	1.0254
25	2.5	8	1,000	27.50	0.0083
26	5	5	10	155.80	0.0416
27	10	5	1	173.50	20.2703

Analysis of variance (ANOVA) was analyzed to identify the parameters and interaction factors that influence the generation of bio-electricity in this study. The ANOVA results are shown in **Table 3**. Important factors that affect bio-electricity by reviewing the response voltage obtained are indicated by the p -value value of less than 5 % (p -value < 5 %) and the results obtained that in **Table 3** shows the independent parameters in the form of time (B) and external resistance (C) have a significant effect. In addition, the interaction between molasses concentration and time (AB) as well as time and external resistance (BC) also has a significant effect because it has a p -value < 5 % (0.05).

Table 5 Analysis of variance (ANOVA) for voltage response results from quadratic model.

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	1,289E + 05	17	7,580.50	21.29	< 0.0001	significant
A-Concentration of molasses	653.58	1	653.58	1.84	0.2053	
B-Time	10,085.93	1	10,085.93	28.33	0.0003	
C-External resistance	55,304.67	4	13,826.17	38.84	< 0.0001	
AB	6,315.61	1	6,315.61	17.74	0.0018	
AC	2,206.76	4	551.69	1.55	0.2612	
BC	5,659.87	4	1,414.97	3.97	0.0349	
A ²	57.05	1	57.05	0.1602	0.6973	
B ²	27,407.50	1	27,407.50	76.99	< 0.0001	
Residual	3,560.04	10	356.00			
Lack of fit	3,560.04	5	712.01			
Pure error	0.0000	5	0.0000			
Cor total	1,324E + 05	27				
<i>Std. Dev = 18.87; R² = 0.9731; Adeq. Precision = 13.1535</i>						

The analysis given to the ANOVA results for the voltage response gives a significant model characterized by a *p*-value less than 0.05. In addition, the significance of the model is supported by data from the Model F-value of 21.29. Although the results of this ANOVA only provide a few factors that have a significant influence including, B, C, AB, BC, B², while the review of the level of conformity between the experimental results and the given model can be seen from the value of R² which gives a value of 0.9731. Based on this value, the value of R² is close to 1, so it can be said that the model obtained can be used to represent the experimental results and predict or determine the voltage from the results of this study. Adeq review. Precision can also provide an illustration that the model obtained is capable of being used as a reference in optimizing factors in producing the optimum voltage, this is because Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 13,153 indicates an adequate signal. This model can be used to navigate the design space. After that, the optimum voltage can be obtained from the experimental design that has been carried out using the model Eq. (1).

$$\begin{aligned}
 \text{Voltage} = & 135.22 - 3.36*A + 30.70*B + 58.09*C_{1\text{ k}\Omega} + 17.19*C_{10\text{ k}\Omega} + 34.50*C_{100\text{ k}\Omega} - \\
 & 39.45*C_{1,000\text{ k}\Omega} + 31.50*AB - 10.66*AC_{1\text{ k}\Omega} + 14.21*AC_{10\text{ k}\Omega} - 16.43*AC_{100\text{ k}\Omega} + \\
 & 5.61*AC_{1,000\text{ k}\Omega} + 16.04*BC_{1\text{ k}\Omega} - 21.08*BC_{10\text{ k}\Omega} + 31.17*BC_{100\text{ k}\Omega} - 27.98*BC_{1,000\text{ k}\Omega} \\
 & - 4.01*A^2 - 83.59*B^2
 \end{aligned}
 \tag{1}$$

Table 6 Analysis of variance (ANOVA) for power density response results from quadratic model.

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	1,172.96	17	69.00	5.54	0.0044	Significant
A-Concentration of molasses	4.08	1	4.08	0.3279	0.5795	
B-Time	32.86	1	32.86	2.64	0.1353	
C-External resistance	885.81	4	221.45	17.79	0.0002	
AB	31.73	1	31.73	2.55	0.1415	
AC	45.53	4	11.38	0.9143	0.4923	
BC	112.53	4	28.13	2.26	0.1349	
A ²	75.11	1	75.11	6.03	0.0339	
B ²	30.21	1	30.21	2.43	0.1504	
Residual	124.49	10	12.45			
Lack of Fit	124.49	5	24.90			
Pure Error	0.0000	5	0.0000			
Cor Total	1,297.45	27				
<i>Std. Dev = 3.53; R² = 0.9041; Adeq. Precision = 13.1535</i>						

Based on **Table 6**, it is shown that the model obtained is significant with a *p*-value less than 0.05 (*p*-value = 0.0044) and the Model F-value of 5.54 implies the model is significant. In this case, the significance

of the model is only given to C and A² because the *p*-value shows less than 0.05. Meanwhile, independent parameters and other interactions have a significant effect because the value is more than 0.1. If there are many insignificant model terms (excluding those needed to support the hierarchy), reducing the parameters can improve the model. Even though there are some insignificant parameters, the model can still be used to optimize the parameters. This is indicated by the R² value of 0.9041 (closer to 1) and adeq. Precision is worth 13.1535 (greater than 4 is desirable). Furthermore, the condition in terms of coded variables can be utilized to form expectations around the reaction for given levels of each calculate. By default, the high levels of the components are coded as +1 and the low levels are coded as -1. The coded condition is valuable for distinguishing the relative affect of the variables by comparing the parameter coefficients.

$$\begin{aligned} \text{Power density} = & 1.80 - 1.12*A + 1.99*B + 11.42*C_{1\text{ k}\Omega} - 2.22*C_{10\text{ k}\Omega} - 2.80*C_{100\text{ k}\Omega} - \\ & 3.06*C_{1,000\text{ k}\Omega} + 2.23*AB - 2.20*AC_{1\text{ k}\Omega} + 1.51*AC_{10\text{ k}\Omega} - 0.81*AC_{100\text{ k}\Omega} \\ & - 0.94*AC_{1,000\text{ k}\Omega} + 5.04*BC_{1\text{ k}\Omega} - 1.6*BC_{10\text{ k}\Omega} - 0.76*BC_{100\text{ k}\Omega} - 4.42*BC_{1,000\text{ k}\Omega} \\ & + 4.61*A^2 - 2.78*B^2 \end{aligned} \quad (2)$$

After optimization of the determined factor parameters, the conditions for obtaining optimum bio-electricity (in terms of voltage and power density) using microbial fuel cells with type i-optimal response surface methodology are at concentration of molasses 2.5 %; external resistance 1 k Ω for 5.7 weeks with a voltage of 201.34 mV/m² and a power density of 22.24 mW/m².

Conclusions

The most optimum external resistance to produce the largest bio-electricity is 1 k Ω . The combination of Sidoarjo mud medium with the most optimum molasses addition is 2.5 %. The largest electric voltage generated 210.25 mV/m², electric current of 51.75 mA/m², and power density of 38.91 mW/m². The addition of dominant bacteria *Shewanella oneidensis MR-1* and *Escherichia coli* as starter inoculum is not effective in producing bio-electricity. Bacteria in Sidoarjo mud have more potential as a culture source than with the addition of dominant bacteria. In addition, a review of the carbon cloth electrode content was carried out using SEM-EDX and it was found that there was a change in the carbon composition before and after the microbial fuel cells process was run for 9 weeks. Significant changes in composition can be seen from the carbon content before operation which was 97.27 % and while after 9 weeks the carbon content was reduced to 0.67 % and there were other contents such as Sulfur, Silica, Ferrum, Phosphorus and other elements caused by bacterial metabolism in biochemical degradation of organic components.

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