## Electrical Characteristics of Chitosan-Carrageenan Membrane Implementation and Salt Bridge in Microbial Fuel Cell Using Yeast Fermented Cassava Waste Substrate

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### Abstract

A Microbial Fuel Cell (MFC) is an electrochemical system that generates energy utilizing waste as a substrate and the results of microbial metabolism processes. This research utilizes yeast fermented cassava waste as a substrate to determine the electrical characteristics of PEM in the form of a chitosan-carrageenan membrane and salt bridge. The cassava waste is from the waste produced in the manufacture of tapioca flour. A dual-chamber MFC made of acrylic with a size of 8x8x10 cm is used. Cassava waste substrate with carbon electrodes would be in the anode compartment, and seawater electrolyte with Cu(Ag) fiber electrodes would be in the cathode compartment. Each measurement holds ±250 ml in each compartment. The MFC system consists of 10 cells and is analyzed every hour for 120 hours using a multitester. According to the results of the research, cassava waste (liquid and onggok) can be used as a substrate in the MFC system, which has the potential to produce alternative electrical energy. Compared to salt bridges, the use of PEM in the form of chitosan-carrageenan membranes produces more significant and better electrical characteristics. However, the chitosan-carrageenan membrane is still less suitable in the long term than the salt bridge.

### 1. Introduction

A microbial Fuel Cell (MFC) is a device for converting chemical energy into electrical energy through the catalytic activity of microorganisms (Chae et al., 2008). MFC is the same as a fuel cell consisting of an anode, a cathode, and an electrolyte. However, the MFC as an anode component is used for microbial culture in microbial metabolic activity (e.g., microbial consortia that oxidize organic substrates such as glucose). The principle of MFC is in the form of microbial activity in the liquid medium. Microbial activity can produce organic components containing hydrogen elements such as ethanol, methanol, and methane gas that can be used to produce electrons and electric current
The MFC system can also be influenced by several factors, including using substrates, electrodes, and proton exchange membranes (PEM).

The intermediate substrate MFC research uses household and food industry organic waste. The following is the study of Utari et al. (2014), who researched fruit waste using MFC technology and adding yeast and acetate variations. The same year, Utari, Hermayanti, and Nugraha’s research (2014) used tofu industrial wastewater using the salt bridge method in MFC. Using waste as a substrate in MFC research can be pretty efficient as a source of growth for microorganisms because organic materials are contained in the waste, especially in the food industry. One of the many wastes produced in Indonesia, especially in Lampung Province, is industrial cassava waste. The cassava industry produces liquid and solid waste (onggok) in tapioca flour (Rukmana, 1997). Tapioca flour processing produces waste (solid and liquid) of about 2/3 to 3/4 parts or about 75% of the raw material. (Yohanista et al., 2014).

Tapioca liquid waste contains organic matter, including glucose at 21.067%, carbohydrates at 18.900%, and vitamin C at 51.040% (Sumiyati, 2009). While the remaining starch causes the onggok waste to have a relatively high carbohydrate content of 50-70%, so it can be used as a substrate or growth medium for microorganisms (Fitrilyani, 2010).

Until now, many studies have examined the performance of MFCs to produce better electrical potential, such as in terms of MFC reactor configuration, types of electrolytes, electrode materials, and microorganism cultures. Zahara (2011) has researched MFC by utilizing Saccharomyces cerevisiae culture, which can be used in MFC technology to obtain electrical energy. Culture Saccharomyces cerevisiae is a type of yeast microorganism usually found in yeast or the scientific language yeast. Saccharomyces cerevisiae cells can grow in a medium containing high concentrations of sugar water. Sugar compounds produced by cellulosic microorganisms for their growth can be utilized by Saccharomyces cerevisiae. This species can ferment various carbohydrates and produce invertase enzymes that separate sucrose into glucose and fructose and convert glucose into alcohol and carbon dioxide (Agustining, 2012).

Another supporting factor that can affect the performance of the MFC system is the use of proton exchange media. Proton exchange media is a medium that separates the anode and cathode compartments in the MFC system (Novitasari, 2011) and is also a place for protons to diffuse from the anode to the cathode. The proton exchange media used in the MFC system are proton exchange membranes (PEM) and salt bridges (salt bridges). Proton exchange media generally used in MFC systems, especially in Indonesia, are salt bridge types. The salt bridge is easy to obtain and has a very affordable price.

Meanwhile, other proton exchange media with PEM type commonly used in research are Nafion and Ultrex CMI-7000. The results of using this type of PEM produce a better electrical potential when compared to a salt bridge. However, the price of Nafion membranes can be said to be expensive, which makes some researchers look for cheaper alternatives. Currently, there are several studies on PEM, which can be said to be an efficient and affordable alternative to PEM. One is the study of Ibrahim et al. (2020), who tested chitosan and carrageenan materials with a mixture of PVA, which would later become membranes. The results of this study were the highest in the 1:1 treatment (Ibrahim et al., 2020). The development of the MFC system continues to this day, both in selecting electrodes, substrates, microorganism culture, and proton exchange media such as PEM and salt bridges which can produce the best electrical potential.

In connection with the above explanation, a study was conducted to analyze the effect of yeast-fermented cassava waste (liquid and onggok) as a substrate with the use of PEM made from chitosan-carrageenan and the use of salt bridges on the electrical characteristics of the MFC system performance. The MFC system in this study consisted of 10 cells arranged in series with seawater electrolytes and a pair of Cu(Ag-C) electrodes. The microbes obtained came from adding yeast (yeast) to instant bread and accelerated the fermentation process in cassava waste. The results of this study are expected to be used as an efficient alternative electrical energy with the selection of MFC components with better performance.

2. Research methods

The tools used in this study include an MFC system container made of acrylic with a thickness of 1.5 mm, a 3 cm diameter PVC pipe, a glass container, a hot plate magnetic stirrer, a measuring cup, a seawater filter, and a digital multimeter. While the materials used, include cassava waste (liquid and onggok), instant yeast of the brand Permipan, seawater, Cu(Ag) fibers, carbon rods, chitosan, carrageenan, PVA, agar-agar, equates, steearic acid, acetic acid, 96% alcohol, solution of AgNO₃, and HNO₃. This research method was carried out in several stages, including electroplating Ag on Cu, making PEM, preparing cassava waste substrate, designing and manufacturing MFC systems, and system testing and data collection.

2.1 Electroplating Process of Ag on Cu

The process of electroplating Ag (silver) on Cu (copper) using an electrolyte solution in the form of a silver-plated solution (AgNO₃) as much as 300 ml. In the electroplating process, the electrodes are Cu fibers as the cathode and carbon rods as the anode. The surface of the Cu fibers is cleaned first with a 1% HNO₃ solution before starting electroplating. It is to reduce the fat content attached to Cu. After cleaning with a 1% HNO₃ solution, the Cu fibers were cleaned with 96% alcohol to remove the HNO₃ content attached to the Cu. The electroplating process is carried out with a voltage of 2 volts for 5 minutes (Rizki, 2019).

2.2 PEM Making Process

Chitosan-Carrageenan Membrane

Chitosan-carrageenan membrane begins with dissolving 2% (w/v) chitosan by dissolving it in acetic acid and dissolving 1% (w/v) carrageenan by dissolving it in distilled water at a temperature of 70°C. Next, the chitosan solution was mixed with the carrageenan solution with a chitosan-carrageenan ratio of 1:1 (v/v). The mixed solution was then...
added with 5% (v/v) PVA. The solution was stirred manually using a small spatula until homogeneous. The homogeneous solution was then poured into a petri dish mold and dried at 70°C for 12 hours in the oven to obtain a dry chitosan-carrageenan film. The membrane was then removed from the mold by immersion in 1% KOH for 10 minutes. The membrane was then rinsed with distilled water until the pH was neutral (Ibrahim et al., 2020). Then, make a solution of 2% stearic acid in 100 ml of 96% alcohol. Stir until homogeneous using a magnetic stirrer. After mixing the solution, the chitosan-carrageenan membrane was soaked with a stearic acid solution for up to half a day. Furthermore, the membrane was dried using an oven at a temperature of 70°C for ±3 hours.

**Salt Bridge**

Filtering seawater before being mixed with agar powder aims to remove particulates contained in seawater (Susanto, 2020). The salt bridge in this study used agar mixed with seawater in a ratio of about 600 ml for every 7 grams of agar powder. Mixing the two ingredients is done by heating it on the stove with continuous stirring until homogeneous. After the solution is homogeneous, the solution is printed on a 3 cm diameter pipe with a length of 2 cm.

### 2.3 Preparation of Cassava Waste Substrate

Cassava waste is in the form of liquid waste and solid waste or is called onggok. Generally, the waste is generated from making tapioca flour both on an industrial and home scale. The cassava waste used in this study was obtained from its processing by peeling, washing, grating, squeezing, and settling. In this process, onggok waste is obtained from the extortion and liquid waste from the deposition. To be used as a substrate, onggok and liquid waste are mixed using a blender so that the resulting substrate becomes like a slurry. It then adds about 1% baker’s yeast to the substrate to produce microorganisms in the waste.

### 2.4 MFC System Design and Manufacturing

The MFC system design uses a dual chamber type with a chitosan-carrageenan membrane separator and a salt bridge. The two compartments will be made up of 10 pieces arranged in series. The anode compartment contains cassava waste substrate in the form of onggok and liquid using a C (carbon) electrode with a diameter of 4 mm and a length of 5 cm. While the cathode compartment contains an electrolyte solution in the form of seawater with a copper (Cu) electrode with silver (Ag) electroplating so that it becomes a Cu (Ag) electrode. The sea water is filtered first with a water filter to filter organisms contained in seawater. The copper (Cu) used is in the form of fibers with a length of 35 cm, folded in five folds with a size of 7 cm. The container uses acrylic material of 8x8x11 cm, which can accommodate about ±250 ml for each compartment. The anode compartment is made in a closed state or under anaerobic conditions, while the cathode compartment is made in an open (aerobic) condition. Then to separate these two compartments, two types of PEM are used: a chitosan-carrageenan membrane and a salt bridge that will be placed between the compartments. The PEM placed between the compartments is 3 cm in diameter. The MFC system design was first made with the Sketchup 2020 application, as shown in **Figures 1 and 2.**

*Figure 1. MFC system design in series*
2.5 System Testing and Data Retrieval

System testing connects each pair of compartments in a series of 10 cells with connecting cables. The test of this MFC system uses cassava waste (mixed and mashed liquid waste) to which 1% baker’s yeast has been added to the effect of using proton exchange media in the form of a chitosan-carrageenan membrane and a salt bridge. Measurements were carried out every 1 hour for 120 hours (5 days). The circuit used in this study is an open circuit that does not use a load, both LED lights and resistors. Data collection in this study used a digital multimeter as a measuring instrument for electrical characteristics in the form of voltage and current—observational data taken in the form of a current (I) and voltage (V). In addition, observational data in the form of internal resistance (R<sub>in</sub>) and power (P) were obtained from the calculations using Equations 1 and 2.

\[
R_{\text{in}} = \frac{V}{I} \quad (1)
\]
\[
P = V \cdot I \quad (2)
\]

where \(R_{\text{in}}\) = internal resistance (\(\Omega\)), \(V\) = voltage (volts), \(I\) = current (amperes), and \(P\) = power (watts).

3. Results and Discussion

3.1 MFC System Realization

The manufacture of the chitosan-carrageenan membrane as one of the components that affect the performance of the MFC system has been realized. In this study, using chitosan-carrageenan membranes in the MFC system could not withstand water. The chitosan-carrageenan membrane was easily destroyed when the substrate and seawater electrolyte were introduced into the MFC system. Chitosan-carrageenan membranes are still vulnerable to liquids, especially when a shock causes the membrane to be destroyed and cannot be reused. One of the reasons for this is that the quality of the materials in the manufacture of the membrane is not good, which can affect the quality of the membrane itself. Then, to overcome this problem, the chitosan-carrageenan membrane was added to make this membrane hydrophobic (substances that do not dissolve in water), namely, stearic acid. In research, Liu et al. (2020) have used stearic acid to produce superhydrophobic magnetic polyurethane sponges. In this study, stearic acid was used using a coating technique by immersing a sponge in a stearic acid solution. The results of this study can separate water but can absorb oil. From the results of the use of stearic acid in this study, it can be realized in the MFC system that the membrane strength is better than not coated with stearic acid. It can be seen in Figure 3, which shows the chitosan-carrageenan membrane before and after being coated with stearic acid.
Then, the MFC system with a dual chamber type arranged in a series of as many as ten cells was realized. Each cell has a pair of Cu(Ag)-C electrodes in different compartments. The cathode section contains Cu(Ag) electrodes with seawater electrolytes as much as ±250 ml, while the anode section contains C (carbon) electrodes with as much yeast fermented cassava waste substrate. In each compartment, the condition of the cathode compartment is made open, and the condition of the anode compartment is closed. The container used for the MFC system is made of acrylic with a size of 8x8x10 cm. ±250 ml. In this study, the electrical characteristics produced by the MFC system (voltage and current) were measured using a multimeter and the circuit used was an open circuit, which did not add a load in the form of LEDs or resistors. The following Figure 4 shows the realization of the MFC system.
3.2 Electrical Characteristics Research Results

Voltage Measurement

The results of data collection in the form of measurement of the voltage produced by the MFC system with the addition of chitosan-carrageenan membrane types and salt bridges using an open voltage circuit are displayed in graphical form. Data analysis using graphs shows the relationship between the value of voltage (V) against time which can be seen in Figure 5.
Figure 5 shows that the voltage value has changed successively for 120 hours. The voltage obtained on the chitosan-carrageenan membrane and the salt bridge at the 0-hour measurement experienced relatively high results, namely around 2.57 V and 1.06 V. This happened because the bacteria had adapted to the cassava waste substrate media, which had previously been left standing. Minutes before being put into the compartment. It is called the lag phase (adjustment), a period of adjustment to the new environment. Then at the 1st hour to the 6th-hour measurement, there was an increase caused by the ability of active microorganisms to divide and synthesize cell material that took place rapidly, so that the number of these bacteria allowed to increase the number, which resulted in faster metabolic processes and more excellent electrical results. It is referred to as the exponential (splitting) phase (Ardi, 2020). After that, in the next hour until the 120th hour, the voltage decreased and increased, which could be said to be in the stationary phase, i.e., the bacterial growth phase did not increase anymore, or it can be said that the number of bacterial cells that divide with the number of dead is the same (Febriansyah, 2011). It is the same as that experienced by the study of Safitri et al. (2020), which experienced fluctuations in the voltage value during the observation process.

The results of the stress value using a chitosan-carrageenan membrane compared to a salt bridge have a relatively far comparison when seen in Figure 5. At the beginning of the measurement (hour 0), it was seen that the voltage value produced by the chitosan-carrageenan membrane was 2.57 V compared to the salt bridge of 1.06 V. This is because the thickness/volume of PEM can affect the performance of the MFC in transferring H ions, from the anode to the cathode and causes the distance between the cathode and anode to increase, thus affecting the distance traveled by electrons from the anode to the cathode (Rekotomo, 2012). So, the greater the separation distance between the anode and cathode, the lower the resulting voltage will be (Cheng et al., 2006). The value of the chitosan-carrageenan membrane voltage from the 1st hour to the 9th-hour measurement increased and decreased by 2.57 V and 1.99 V, and at the 10th hour to the 24th-hour measurement experienced a relatively high increase of 2.08 V to 3.36 V. After that, in the next hour until the 120th hour, it experienced a state of fluctuation, namely experiencing a continuous increase and decrease in voltage until it reached the final measurement of 0.88 V. Meanwhile, the salt bridge at the third hour 1 to 66 hours experienced a decrease in voltage in a row, namely 1.47 V to 0.96 V and an increase in voltage from 67 hours to 120 V hours, which was 1.12 V to 1.35 V.

From the voltage values obtained, the final results were quite different, where the use of chitosan-carrageenan membrane at the 30th hour began to experience a continuous decrease when compared to the salt bridge at the 67th hour, which experienced a continuous increase. Although the change in the voltage value of the chitosan-carrageenan membrane is not good compared to the use of a salt bridge, the maximum voltage value produced by the use of the chitosan-carageenan membrane is 3.36 V, and the salt bridge is only 1.56 V. One of the performance factors of the MFC system is the diffusion of oxygen from the cathode to the anode. According to Min et al. (2005), oxygen can diffuse into the anode chamber at a maximum speed of 0.014 mg/hour on the MFC membrane, but the diffusion of oxygen into the anode chamber on the salt bridge was not detected. The effect of the diffusion of dissolved oxygen into the anode chamber is likely to vary as a function of the bacteria or microbial community present in the system, which may contribute to the ability of the bacteria to breathe or scavenge for oxygen. Thus, oxygen diffusion into the anode chamber will affect the electricity generated (Min et al., 2005).

**Current Strong Measurement**

In addition to measuring the voltage, on the other hand, there is a substantial value of the current generated in the MFC system. The results of observations in the form of the relationship between the value of the current strength (i) and the time taken up to 120 hours are shown in the form of a graph that can be seen in Figure 6.
Figure 6. The relationship between the value of the current strength with time

The graph in Figure 6 shows the maximum current value for the chitosan-carrageenan membrane of 0.077 mA at the 2nd hour, while the salt bridge experienced a prolonged increase in the process of 0.036 mA at the 93rd hour. Then, when the 3rd-hour measurement decreased, the value of the current strength increased again at the 11th hour and decreased again. The graph continues to fluctuate. It is due to the inconsistency of the ions from the anode chamber flowing to the cathode chamber in the PEM, disrupting the ion transfer process that affects the system's electricity. Another thing is also caused by the ability of the electrodes used in the anode chamber to carry current not optimally because it allows the formation of biofilms on the electrodes produced from living bacterial cells and dead bacterial cells, which can form a layer on the electrode surface (Kim et al., 2007). In addition, another thing that is a factor in the value of the current strength fluctuating can be caused by the presence of H⁺ (hydrogen) metabolic products at the anode, which causes the longer the concentration of H⁺ increases, the longer it will cover the surface of the electrode at the anode so that the electron transfer process from bacteria to the electrode is not maximized (Trinh et al., 2009).

After that, at the 45th hour, the graph shows that the change in the current strength of the chitosan-carrageenan membrane is inversely proportional to the salt bridge. The chitosan-carrageenan membrane continued to experience a decrease in the current strength value until it reached a value of 0.017 mA at the 120th hour, while the salt bridge continued to experience an increase in the current strength value until it reached a value of 0.029 mA at the 120th hour. I.e., the value of the current (I) produced is directly proportional to the voltage (V) value. The greater the value of the resulting voltage, the greater the electric charge that can be transferred so that the value of the strong current will also be more significant and vice versa. It is also because, as is known from the explanation of the voltage measurement, the effect of oxygen diffusion from the cathode to the anode chamber can affect the electrical ability of the MFC system. As in the study of Min et al. (2005), it was stated that the use of membranes experienced speedy oxygen diffusion compared to the use of salt bridges where oxygen diffusion was not detected, and the oxygen permeability of the membrane could reduce the electrical ability of the MFC system (Min et al., 2005).

**Internal Resistance Measurement**

The measurement of internal resistance ($R_{in}$) is obtained from the results of calculations using Equation 1. The data is displayed in the form of a graph shown in Figure 7.

Figure 7. The relationship between the value of internal resistance to time
The graph in Figure 7 shows that the value of internal resistance \( R_{\text{in}} \) on the use of yeast fermented cassava waste as a substrate with the addition of a chitosan-carrageenan membrane and a salt bridge as PEM resulted in a maximum \( R_{\text{in}} \) value of 230 kΩ at 11 hours on the chitosan-carrageenan membrane. And 350 kΩ at the 26th hour on the salt bridge.

The value of \( R_{\text{in}} \) using a chitosan-carrageenan membrane is smaller, which is 49.423 kΩ, compared to the salt bridge of 70.667 kΩ at the 0-hour measurement. It can be influenced by using PEM thickness/volume in the MFC system. The distance between the anode and cathode, which is increasingly vulnerable, affects the transfer of electrons and \( \text{H}^+ \) ions resulting in internal resistance in the MFC system (Rekotomo, 2012).

It can be seen from the graph that after 0 hours, both the chitosan-carrageenan membrane and the salt bridge experienced fluctuations. However, with the use of chitosan-carrageenan membranes, the value of \( R_{\text{in}} \) tends to be more stable than the use of salt bridges. It is because the voltage and current strength results show that the chitosan-carrageenan membrane is larger than the salt bridge. It also follows the value of the voltage and current obtained based on Ohm’s Law; the resistance value is inversely proportional to the value of the voltage and current. Then, at 43 hours, the \( R_{\text{in}} \)-value of the chitosan-carrageenan membrane became more significant than in the salt bridge. It is because the oxygen permeability of the membrane is the cause of the significant resistance obtained which results in a decrease in the generated electricity (Min et al, 2005).

**Power Measurement**

In addition to the value of \( R_{\text{in}} \), calculations were carried out to find the value of power (P) obtained by the MFC system in this study. The calculation of the power value uses Equation 2, the results of which are displayed in the form of a graph shown in Figure 8.

![Figure 8](source)

Figure 8. The relationship between the value of power to time

Figure 8 shows the power value against time using chitosan-carrageenan membrane and salt bridge as PEM. The graph above shows that the power value at hour 0 on the chitosan-carrageenan membrane is much greater, namely 0.134 mW, compared to the salt bridge, which is very small, which is 0.016 mW. However, the chitosan-carrageenan membrane’s power results are pretty good compared to the salt bridge. The maximum power value generated by the chitosan-carrageenan membrane reached up to 0.245 mW at the 2nd hour, and the salt bridge reached up to 0.055 mW at the 93rd hour.

4. **Conclusion**

yeast - fermented cassava waste (liquid and onggok) in the MFC system resulted in electrical characteristic values for each measurement of voltage and current values. It can be said that cassava waste (liquid and onggok) can be used as an alternative to renewable energy. Then, the chitosan-carrageenan membrane produced a higher and better electrical characteristic value than the salt bridge. However, the chitosan-carrageenan membrane is still less suitable in the long term than the salt bridge. Different types of PEM, PEM sizes, and PEM materials can affect the electrical characteristics produced.

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