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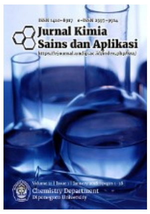

Utilization of Ecoenzyme *Citrus reticulata* in a microbial fuel cell as a new potential of renewable energy

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Abstract

The world's energy needs generally come from non-renewable sources. In other circumstances, some research on renewable energy is being developed from a variety of different sources, one of which is biomass. Biomass changes the energy system towards the modernization of the bioenergy system by utilizing the concept of biochemical systems (BESs). A microbial fuel cell is known as one of the renewable technologies that convert biomass with the help of microbes to produce electricity. This research is based on a microbial fuel cell based on ecoenzyme *Citrus reticulata* known as Ecoenzyme fuel cell to determine the electrical value (voltage, current, and power density). Ecoenzyme was made from orange peel waste, molasses, and water with a ratio of 3: 1: 10 and fermented for a month. Meanwhile, the electrode device used was a pencil graphite. Some treatments were carried out to optimize the performance of the system based on the number of electrodes (one pair and three pairs), and the stirring conditions, stirring at 200 rpm and without stirring (0 rpm). The EFC system was run for four weeks (600 hours) with the highest voltage obtained at 650 mV and current at 29.55 mA. The ecoenzyme produced the most significant power density of 750 W/m^2 in the treatment of 3 electrode pairs with a stirring speed of 200 rpm. The influence of electrodes and stirring in the MFC system upsurged the electrical value output by 53.7% for a pair of electrodes and 142% for three electrode pairs. Further development will continue to be done to improve the performance and output of the Ecoenzyme fuel cell system as a future renewable energy source in Indonesia.

1. Introduction

Energy is an essential factor in all aspects of human life, which is increasing every year [1]. The National Energy Outlook [2] shows that Indonesia in 2015 produced 2,848,025,000 BOE (Barrel Oil Equivalent) energy and had to import an energy supply of 348,267 BOE. This data is based on assumptions from the growth of gross domestic product (GDP), which shows that the energy needs of the Indonesian people have increased 1.8 times with an average growth of 6.4%. This shows that the availability of energy becomes essential [3]. So those energy problems are sought to be overcome by the movement of energy supply to the biomass sector [4].

Biomass changes the energy system towards the modernization of the bioenergy system [5], which triggers a change in the direction of future energy welfare. Biomass is utilized through the concept of biochemical systems (BESs) by converting biomass components into electrical energy through the use of microorganisms [6]. The direction of the movement of renewable energy production in Indonesia has utilized the BESs concept, including a microbial fuel cell (MFC) system.

MFC is a renewable energy technology that is being developed for the future. This technology utilizes the catalytic activity of microorganisms to oxidize the substrate in anaerobic conditions (at the anode) and produce electrons that are flowed into the cathode compartment to produce electrical energy [7]. Along with the development, optimization of energy production leads to the development of the MFC substrate. One of the potential sources developed for the development of microbial fuel cells in the future is Ecoenzyme.

In this study, Ecoenzyme was produced from the processing of organic waste utilizing the waste of Medan tangerine (*Citrus reticulata*), molasses, and water with a component ratio of 3: 1: 10. Organic waste was stored for three months to produce a liquid solution. Ecoenzyme produces organic acids in the form of lactic acid and acetic acid as side products [8]. Molasses in solution will act as a source of carbon and organic acids for the fermentation process that enters the glycolysis pathway or Embden Mayerhof Parnas (EMP). Organic components and metabolic processes that occur involve the role of microorganisms as exoelectrogens [9].

Electric production with the ecoenzyme fuel cell system further utilizes and optimizes the fermentation process in *Citrus reticulata* Ecoenzyme. This concept is the same as MFC, which utilizes the catabolism of organic acids by microorganisms. Organic acids will be used by organisms to produce electrons and protons [10]. The more organic acid, the more electrons are produced so that energy production is higher. Based on the description, in this study, the ecoenzyme was used as a substrate in the anode compartment to determine its potential as a substrate in MFC. The MFC system performance test was then performed on the variation of the number of electrodes and treatment with and without stirring.

2. Methodology

The working principle of MFC based on ecoenzyme *Citrus reticulata* is simple. Ecoenzyme C.reticulata acted as an anode, and electrolyte solution acted as a cathode. The anode and cathode compartments were separated by cellulose acetate membrane.

2.1. Equipment and Materials

The equipment used is glassware, multimeters, analytical scales (KENKO Panasonic Kenko Electric Model), knives, cellulose acetate membrane (Sartorius Biolab Products), Hot Plate (Wisd Laboratory Instrument IUSH 20 D), aluminum foil, pH meter (HANNA pH 21 pH/mV meter), Digital Refractometer (0-21)%, acrylic glue, graphite rod (pencil), resistor, filter paper, and magnetic stirrer. The materials used in this study consisted of Citrus reticulate skin waste originating from Medan, phosphate buffer pH 7.0 (NaH_2PO_4, and $Na_2HPO_4.12H_2O$), molasses (sugar waste), distilled water, $KMnO_4$ (powder) and vaseline.

2.2. Electrode preparation

The electrodes used were graphite from a pencil. The electrode preparation stage was carried out by washing graphite electrodes with distilled water until a neutral pH (pH = 7) obtained.

2.3. Preparation of potassium permanganate solution ($KMnO_4$)

Potassium permanganate solution ($KMnO_4$) was prepared by dissolving 15.8 grams of $KMnO_4$ in 500 mL distilled water and heated for 15 minutes. Then, the solution was stored in a dark bottle and a dark room without light for two days.

2.4. Preparation of Ecoenzyme *Citrus reticulata* Solution

Ecoenzyme solution was prepared by mixing Medan citrus peel (*Citrus reticulata*) waste from Medan, molasses, and water in a ratio of 3: 1: 10.

2.5. Microbial fuel cell design

The anode and cathode compartments were made of acrylic sheet with a size of 10 cm x 7 cm x 10 cm. Next, in the middle, the cellulose acetate membrane was attached. The anode compartment was filled with an ecoenzyme solution, while the cathode side was filled with a 0.2 M KMnO4 solution. The microbial fuel cell system model based on the ecoenzyme *Citrus reticulata* is presented in Figure 1.

2.6. Measurement of Brix levels of ecoenzyme

1-3 drops of the solution were put into the refractometer. The refractometer used was in the range of 0-21% Brix. The Brix level was determined from the boundary between the blue and white parts in the device lens.

2.7. Ecoenzyme Fuel Cell (EFC) Performance Testing

System performance was determined by measuring the power density produced by the system. The variable determining the performance of EFC was the type of electrode and stirring. Electrodes can be divided into one pair and three pairs of electrodes while stirring was distinguished by stirring (200 rpm) and without stirring (0 rpm). The power density was calculated based on voltage (V), current (I), and electrode surface area (A).

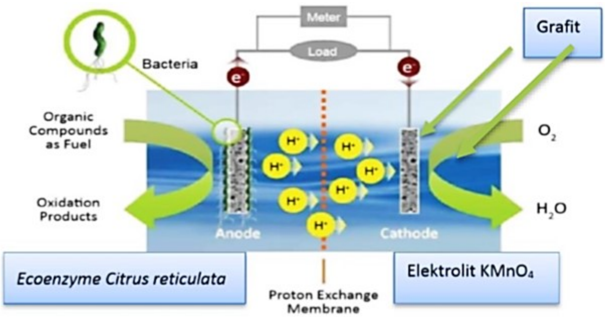

Figure 1. EFC Construction Model based on C.reticulata ecoenzyme

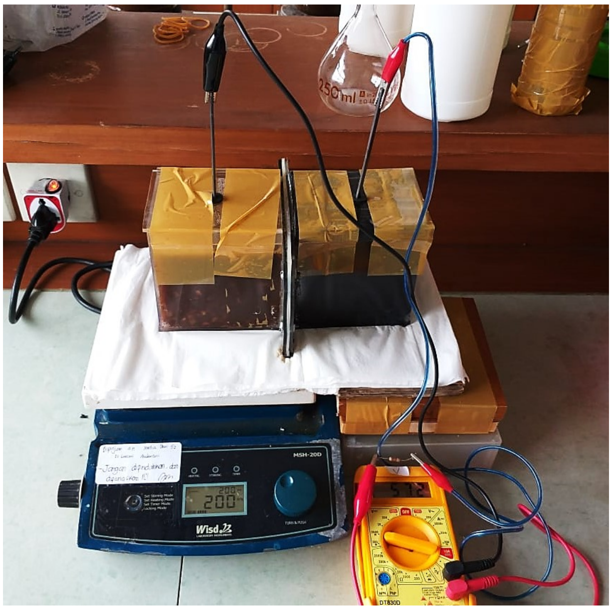

Figure 2. EFC construction in the treatment of one pair of electrodes and 200 rpm stirring

3. Results and Discussion

3.1. Ecoenzyme Fuel Cell

The ecoenzyme fuel cell device is part of a microbial fuel cell (MFC) that utilizes the catabolism of organic compounds by microorganisms to produce electrical energy [7]. During the process, oxidation and reduction reactions occur at the anode and cathode. The anode chamber is filled with ecoenzyme *Citrus reticulata*. Molasses or molasses is a byproduct of the process of making high sugar content of TSAI (Total Sugar as Inverted). TSAI levels of molasses generally range between 50-65% [11]. The presence of molasses in solution will act as a source of sugar and organic acids for the fermentation process that enters the glycolysis pathway or Embden Mayerhof Parnas (EMP).

Glycolysis is the process of breaking down glucose substrate enzymatically into two pyruvate molecules (3 carbon atoms). During operation, glucose will be converted to pyruvate and in the process of metabolism produces electrons. Furthermore, these electrons will reduce the NAD+ complex to NADH. In the metabolic system, NADH acts as an electron carrier. Electron displacement occurs in the process of cellular-level metabolism. In the process of electron transfer in the plasma membrane, NADH bacteria is oxidized to NAD+ and electrons [9]. The electrons are then transferred from the bacterial cell to the electrode and flow through an external circuit to the cathode. Whilst, the H^+ proton produced from the reaction is transferred through a semipermeable membrane. Simply put, the bacterial metabolic reaction in the anode compartment is described in the reaction below.

Anode: $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO2 + 24H^+ + 24e^-$ $\Delta G^o = -1438$ kJ/ mol

Referring to the reaction that occurs, the metabolic reaction of organic compounds in the system gives a change in Gibbs free energy (ΔG^o), which is negative, which indicates that the reaction takes place spontaneously. Thermodynamically a value of ΔG^o less than 0 indicates the level of ease of a reaction that occurs. The smaller the value of ΔG^o, the easier the reshuffle reaction occurs. These results indicate that the metabolic reaction in the Ecoenzyme *Citrus reticulata* solution runs spontaneously so that it can produce electricity [12].

Ecoenzyme is one of the new substrates for the development of microbial fuel cells in the future. Organic components and metabolic processes that occur involve the role of microorganisms as exoelectrogens. Microorganisms in the MFC substrate will act as exoelectrogens to transfer electrons exocellularly. Besides, several other studies mention that microorganisms will act as electrochemically active bacteria, anode respiring bacteria, and electrigens. This group of microbes is commonly found on substrates with high organic material and easy to settle, such as activated sludge, wastewater, marine and soil sediments [13], and ecoenzyme. In this type of substrate, many microbes with high potential (agent of bioelectronics) are found in delivering electrons through external circuits.

The cathode chamber is filled with a 0.2 M $KMnO_4$ electrolyte solution and a phosphate buffer 7. The $KMnO_4$ solution acts as an acceptor that captures electrons from the anode. $KMnO_4$ has a standard reduction potential value of 1.70 V, which is a strong oxidizing agent [14]. The use of KMnO4 in the MFC operating system can increase the value of the system's power density up to 11 times [15]. The reaction in the cathode compartment is explained in the reaction below.

Cathode: $MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O$ [16]

3.2. System Performance based on Number of Electrodes

Measurement of system performance is done by measuring the value of voltage, current, and power density at a different number of electrodes. Electrodes can be divided into one pair and three pairs. The variation of the electrodes aims to determine the right number of electrodes to produce high energy. The EFC system compartment uses a dual-chamber with a size of 7 cm x10 cm x10 cm. Each anode and cathode compartment has a total solution volume of 575 mL. The electrodes used were graphite electrodes from pencils. Measurement of system performance was carried out for 600 hours of observation. The results of the measurement of the performance of EFC systems based on ecoenzyme and $KMnO_4$ electrolytes on the variation of the number of electrodes are presented in Figures 3, 4, and 5.

Figure 3 shows that the more electrodes, the higher the electrical value produced by the system. This happens because an increasingly wider electrode surface influences it. Where the area of the electrode is directly proportional to the number of electrodes. The more surface area and more electrodes that are attached to the system, the higher the electrical value generated by the system. Systems with more electrode counts provide more electrons to attach. Electrons produced from the metabolism of organic compounds attach more to the system with a higher number of electrodes, resulting in a higher electrical value [17]. Higher performance in the system is shown in the system with the treatment of 3 pairs of electrodes. The highest voltage value obtained is 624 mV, with a current of 28.27 mA and provides a power density value of 628 W/m².

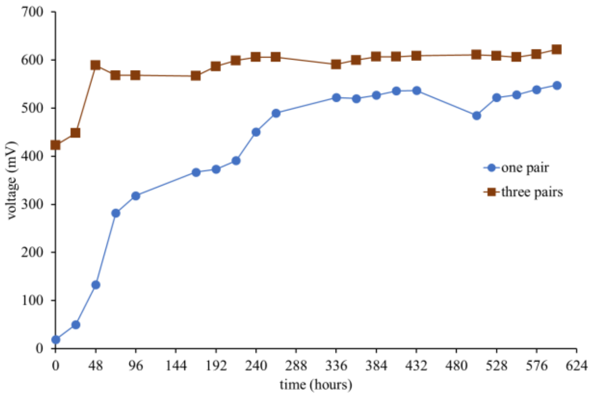

Figure 3. System voltage performance as a function of time and the number of electrodes

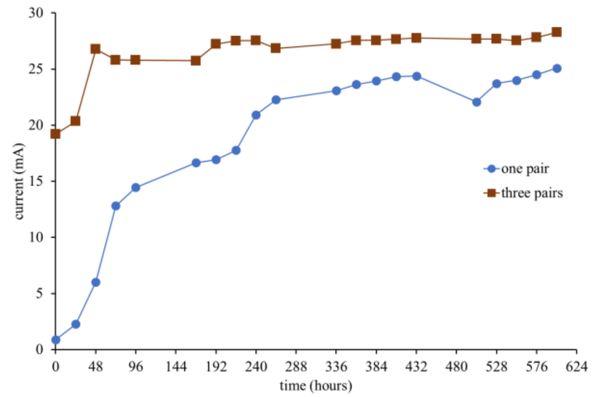

Figure 4. Performance of the system current as a function of time and the number of electrodes

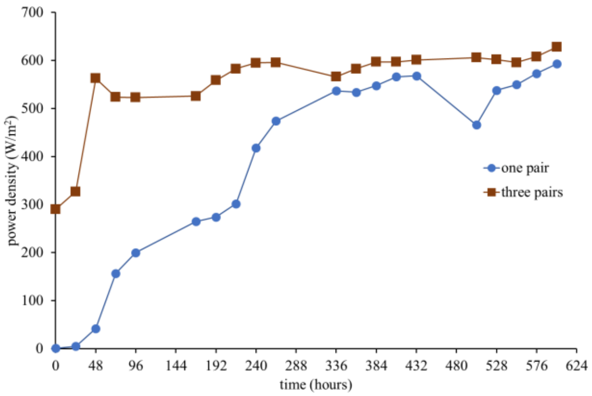

Figure 5. Performance of system power density as a function of time and number of electrodes.

The electrical value produced in this study is higher than in previous studies [18] with the same number of electrodes and compartment sizes. In previous studies, the highest voltage value in the treatment of one pair of electrodes was 389 mV, while the treatment using three pairs of electrodes was 335 mV. These results indicate an increase of 142% in the treatment of one pair of electrodes and 53.7% in the treatment of 3 pairs of electrodes.

Measurement of electrical values (voltage, current, and power density) observed for the treatment of one pair of electrodes shows a cycle that is almost the same as that of 3 electrodes pairs. The slope value profile on the system is influenced by the high and low electrical values generated. Measurement of the electrical value of the system in one pair and three pairs of electrodes has increased sharply from 0 hours to 72 hours and continues to rise until it shows a decrease in the value of the voltage and then rises afterward. An increase and decrease indicate an uncertain metabolic process. This process is influenced by the number of microbes present in the solution. High and low energy generated from the system is strongly influenced by the rate of substrate metabolism by microbes. The quantity of energy produced from the microbial fuel cell system is strongly influenced by the size of the microbes contained in the solution. The higher the size of the microbes used, the smaller the microbial attachment region of the electrode so that the electrical energy produced is also smaller, and vice versa. This phenomenon is also based on the amount of microbial density in solution [19]. In addition, the high electrical energy value is also influenced by the culture of bacterial mixtures in the system [20].

3.3. System performance in stirring and without stirring treatment

System performance was also measured at 200 rpm stirring and without stirring (0 rpm). The stirring speed treatment was carried out on one pair and three pairs of electrodes. Figures 6, 7, and 8 show that the highest voltage value is generated at a speed of 200 rpm by installing three pairs of electrodes. The resulting voltage value is 650 mV. The highest current data is generated from the treatment of 3 pairs of electrodes at a speed of 200 rpm of 29.55 mA, while the highest power density obtained is 750 W/m² produced by the same system.

The treatment of the system with one pair of electrodes at a stirring speed of 200 rpm shows that the movement of the graphic profile is significantly different from the others. The results of the analysis of system performance show that after the observation at 300 hours, the electrical value continues to decrease until the last day of observation, which reaches the lowest voltage value of 166 mV. The decrease in the voltage in this system is caused by two factors, i.e., the stirring speed and the number of microbes in the anode solution. The high stirring speed treatment causes the resulting electrical value to decrease. The stirring process causes the electrons to move continuously; hence the electron contact time with the electrode surface is reduced, which results in a decrease in the value of the resulting electricity. In addition, the condition of the system with one pair of electrodes cannot facilitate the moving electrons to stick to the electrodes. This is because the collision between high electrons is not balanced by the electrode areas where the electrons are attached so that it has implications for the decreasing value of the electricity.

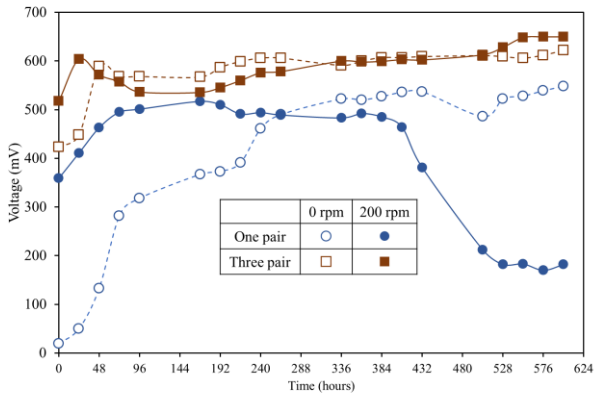

Figure 6. System voltage performance with and without stirring

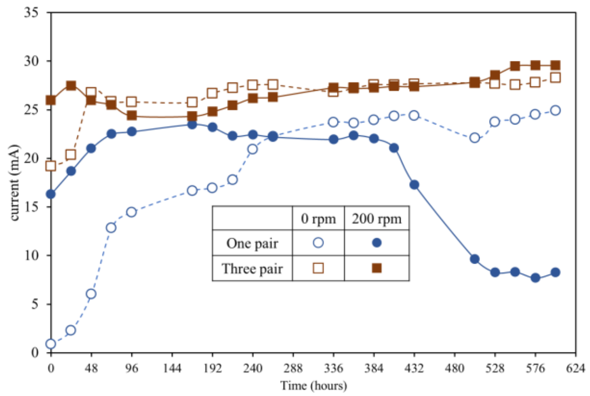

Figure 7. System current performance with and without stirring

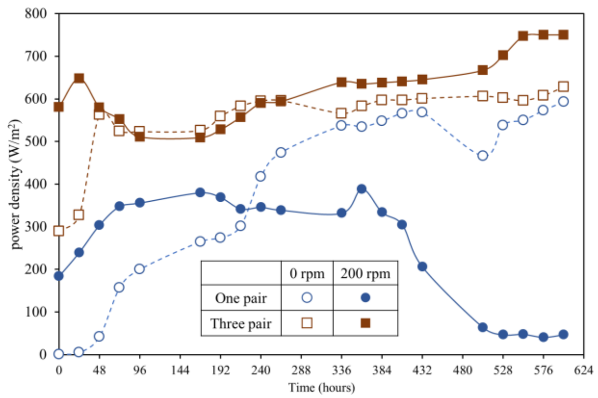

Figure 8. Performance of system power density with and without stirring

Electricity values in the system in the presence of stirring show a higher value compared to previous studies using tofu wastewater substrate and microbial Lactobacillus bulgaricus with a stirring speed of 250 rpm that is equal to 46.1 mV [21]. The results obtained showed that the stirring speed affects the electrical value generated by the system. A system with a 200 rpm treatment produces a higher electrical value compared to a system at 250 rpm. This shows that the higher the stirring speed, the lower the electrical value produced. The value of the power density produced by the system is also higher than in previous studies, which produced a power density value of 31.58 mW/m^2 with cashew apple juice as a substrate [22]. The stirring process influences the difference in the value of the resulting power density in the system. Stirring or stirring speed is very influential on the movement of electrons in the solution. Even the distribution of electrons in the solution can optimize the performance of the system. This is because a more straightforward collision between electrons can increase the performance of the EFC system.

The effect of stirring speed, electron movement, and the resulting electrical value is also influenced by fouling that occurs in the membrane. Fouling is the process of accumulation or deposition of material on the membrane surface [23]. The accumulation of material is influenced by natural organic matter contained in the solution. The formation of fouling on the membrane surface results in a decrease in membrane performance in the proton delivery process so that the electrical value generated by the system without stirring (0 rpm) is lower than the system with 200 rpm treatment.

3.4. Characteristics of Ecoenzyme before and after EFC

The ecoenzyme solution in the anode compartment was measured in pH and Brix levels during system operation. The initial pH of the solution was 4.97 and decreased to 3.45 after four weeks of EFC surgery. The change in pH value in the system affects the catalytic activity of microbes and enzymes in oxidizing the substrate, metabolism, absorption of nutrients and solubility, or the ability of the substrate to oxidize [24].

Table 1. Characteristics of ecoenzyme before and after EFC

Electrode	Acid level (pH)		Brix level	
	before EFC	after EFC	before EFC	after EFC
One pair				
Without stirring (0 rpm)	4.97	3.45	7 %	4 %
Stirring at 200 rpm			7 %	3 %
Three pairs				
Without stirring (0 rpm)	4.97	3.45	7 %	4 %
Stirring at 200 rpm			7 %	3 %

The decrease in pH value in the system is due to the accumulation of H^+ ions, which are donated from bacterial metabolic byproducts such as HNO_3, H_2SO_4, and H_3PO_4. Also, changes in the pH value in the solution also indicate that the higher organic acids produced by microbes in the solution, which also implies that the sucrose content is reduced.

The decrease in the value of sucrose can be known from the measured degree of Brix. Based on the data described in Table 1, the Brix level of the system by electrode variation showed a change from the initial Brix level of 7% and decreased to 4% and 3%, respectively. Treatment with stirring speed variations also showed the same results, i.e., 4% and 3% after EFC treatment without stirring and stirring (200 rpm), respectively. The percentage (level) of Brix in the solution represents a reduction in the concentration of sugar in the solution. The higher the Brix value, the higher the concentration of total sugar in the solution. Decreasing the Brix value in the solution indicates that the total sugar or sucrose in the solution, which acts as a substrate in fermentation for microbes, decreases.

4. Conclusion

Ecoenzyme *Citrus reticulata* has the potential to be developed as a substrate in the MFC system. The highest electrical value generated from the reaction in the ecoenzyme reaches 650 mV, with a current of 29.55 mA and a power density of 750 W/m^2. This result was obtained in the treatment of 3 pairs of electrodes with a stirring speed of 200 rpm after measuring for 600 hours.

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