Optimization of Biogas Liquid Waste from Livestock Manure as a Source of Renewable Energy through Microbial Fuel Cell (MFC) Technology

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Abstract. This study examines the potential of biogas effluent as a source of electrical energy through microbial fuel cell (MFC) technology. The results showed that the one-room MFC produced a maximum voltage of 1302,2 mV, while the two-room MFC with $KMnO_4$ electrolyte reached 1084,3 mV with an electric current of 0,1 mA. Simultaneously, the two-chamber MFC with $K_2Cr_2O_4$ produced a maximum voltage of 1675,8 mV and an electric current of 0,99 mA. This potential, with electrical voltage values equivalent to commercial batteries, can improve the efficiency of electrical energy generation from organic waste and open up wider application opportunities in using renewable energy sources. Characterization of the MFC substrate showed a decrease in organic matter of biogas effluent with COD values of 500,96 mg/L and BOD of 300,23 mg/L. MFC bacteria from biogas waste were dominated by Gram-positive rod-shaped and Sarcina, except for one Gram-negative isolate of Spirillum.

Keywords: biogas, renewable energy, liquid waste, MFC

1. Introduction

The high demand for electrical energy used in industry, transportation, and households in Indonesia is mainly from non-renewable fossil fuels [1]. By 2022, most of the electricity will come from coal (37,6%), natural gas (16,8%), fuel (33,4%) and renewable energy (NRE) (12,2%). This energy crisis

increases with society's and the economy's growth, making energy reserves increasingly depleted. Therefore, efforts are needed to develop renewable energy technologies, including from organic waste [2].

Biogas liquid waste, especially livestock manure sludge, is an organic waste that can be processed into an electrical energy source through microbial fuel cell (MFC) technology [3]. MFC is an electrochemical device that converts chemical energy from organic and inorganic materials into electrical energy through microbial metabolism, thus producing an environmentally friendly energy source [4]. This process generates electrons, which are captured and channelled through the electrodes, creating an electrical potential between the anode and cathode. An MFC system consists of two chambers, the anode and cathode chambers, where the substrate is oxidized and the oxidant (usually oxygen) is reduced [5].

In its development, various forms of organic matter can be used as MFC substrates, such as glucose [6] [4], starch [7] [8], fatty acids [9] [10], amino acids [11], proteins, leachate [9], and organic and inorganic sediments [1] [5]. MFCs have advantages in organic waste treatment and energy independence and are used for small-scale power generation. Despite the challenges of efficiency and durability of microorganisms, research continues for the optimization of this technology, presenting innovative solutions for sustainable energy needs [12].

Biogas effluent from cow dung has great potential as a substrate in MFC technology. The use of cow dung produces a maximum voltage of 0.723V at 50% concentration and is capable of powering a small LED lamp [13]. This research will focus on functionalizing cow dung biogas effluent into a renewable electrical energy source through MFC technology.

2. Methods

2.1. *Tools and Materials*
The tools used in this research are MFC reactor type two-compartment series, digital multimeter, N.Y.A ETERNA cable (1 x 2,5 mm), analytical balance, magnetic stirrer, beaker, measuring cup, erlenmeyer, measuring pipette, and pH meter. The materials used in the study were biogas liquid waste, distilled water, HCl 1 N, NaOH 1 N, standard solution of 5000 ppm glucose, $K_2Cr_2O_7$ 1 N, concentrated H_2SO_4, ferroin indicator, $[Fe(NH_4)_2(SO_4)_2]$ 0,2 N solution, NaCl solution, NH_4Cl, KH_2PO_4, $MgSO_4.5H_2O$, NaOH 40%, H_2SO_4 0,05 N, pure agar, KCl 1 M, KCl 0,01 M and buffer solution pH 7,0.

2.2. *Biogas Liquid Waste Sampling*
MFC substrate samples came from biogas liquid waste in Monapa Village, South Konawe. This effluent, the result of converting livestock manure into biogas, was stored in 5 L jerken and analyzed in the laboratory. This liquid waste becomes the main substrate in the MFC, with microorganisms playing a role in the oxidation of organic matter. Electrons generated during the process are captured by electrodes, generating electrical potential as an energy source to replace fuel oil.

2.3. *Characterization of Biogas Liquid Waste*
The characterization of biogas liquid waste refers to the research of Sudarman et al. [5] and Yao et al. [14], namely C-organic measurements, N-total measurements, COD (Chemical Oxygen Demand) measurements, BOD (Biological Oxygen Demand) measurements, pH measurements (H_2O and KCl) and electrical conductivity measurements.

2.4. *MFC Circuit Manufacturing*
The MFC electrodes used graphite rods from batteries. Previously, treatment was carried out by soaking the electrodes in 1 N HCl and 1 N NaOH, followed by rinsing with distilled water. The electrodes were then soaked again in distilled water before use. Each electrode was perforated and connected to the cable using epoxy and tested with a multimeter to ensure a successful connection. The MFC circuit followed the work of Zaeni et al [1]. Biogas effluent was put into a 500 mL beaker, with a

carbon electrode as the anode, connected to the cathode using a chemical electrolyte. The waste was allowed to stand for 20 hours, and then the voltage and current were measured using a multimeter on the MFC operated in dark conditions and at room temperature. Figure 1 shows the MFC circuit [5].
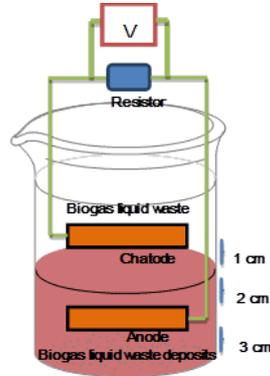
Figure 1. MFC circuit schematic

2.5. *Electrical Energy Measurement*

The electrical energy of the MFC system measured in this study is in the form of electric potential difference (voltage) and electric current. In the initial stage, measurements were taken for 20 hours and recorded every 2 hours. Measurements were made shortly after installation, which aimed to see the maximum open circuit voltage (open circuit voltage/Voc), which is a factor that affects an MFC in producing energy [1] [5].

2.6. *Isolation, Characterization, and Identification of MFC Bacteria*

The bacterial isolation stage consists of several steps: the preparation of liquid and solid media, bacterial inoculation, and bacterial isolation. The enrichment culture media used is modified APW (Alkaline Peptone Water) media [5]. Each litre of modified APW media contains 20 g NaCl, 0,77 g KCl, 0,25 g NH_4Cl, 0,1 g KH_2PO_4, 0,2 g $MgSO_4.7H_2O$ and 2,0 g $NaHCO_3$. The culture medium was then poured into rubber-stoppered tubes and autoclaved at 121°C. The solid media used for bacterial isolation was modified APW media added with pure agar (2%, wt/vol).

Bacterial inoculation in MFC involves taking 1 mL of biogas effluent and putting it into liquid media. The bacteria were incubated for two days at room temperature under dark conditions. After that, dilution and growth of bacteria on solid APW media were carried out. The growing bacterial colonies were isolated using the scratch cup method with an ose onto similar culture media. Characterization of bacterial isolates included observation of colony morphology, cell morphology (shape and Gram staining), and motility test. Bacterial identification was done manually with Bergey's Manual of Determinative Bacteriology.

3. Results and Discussion

3.1. *Characteristics of Biogas Liquid Waste*

After MFC, the biogas effluent changed from black to brown, indicating decreased organic matter levels [13]. Changes in organic matter levels before and after the use of MFC are recorded in Table 1.

Table 1. Characteristics of biogas waste liquid

Testing Parameters	Organic Matter Content	
	Before MFC	**After MFC**
C (%)	2,92	2,20
N (%)	2,01	1,85
C/N	1,45	1,19
COD (mg/L)	1001,92	500,96
BOD (mg/L)	805,54	300,23

DHL (dSm^{-1})	6,00	5,01
pH (H$_2$O)	7,24	7,67
pH (KCl)	6,87	7,54

Table 1 shows that the organic matter content of biogas liquid waste before MFC was 2,92%; after MFC, it decreased to 2,20%. Total nitrogen content before MFC was 2,01%, but after MFC, it decreased to 1,85%. Based on the carbon and nitrogen content analysis, the average C/N ratio of biogas liquid waste is 1,45. The value of the C/N ratio, which is smaller than 15, indicates the process of N mineralization in biogas liquid waste. N mineralization is the process of converting N-organic into N-organic by decomposing microbes. If the C/N ratio is greater than 30, N immobilization occurs; if it is between 15-30, mineralization is balanced with immobilization. The process of mineralization and immobilization of N in sewage is largely determined by the activity of sewage microorganisms, fungi, bacteria, and so on [1] [5].

COD and BOD measurements support the production of electrical energy in MFCs. The higher the COD and BOD values, the greater the energy produced because microbes break down organic compounds, producing ions and electrons as a source of electrical energy. Table 1 shows a decrease in COD and BOD values after using biogas effluent as an MFC substrate, in line with the research of Nosek et al.[10]. Linh and Hai [11] also supported MFCs as BOD sensors, reducing the content of organic compounds in waste.

Table 1 shows the average pH of biogas effluent is around ±7. The pH range of 6,5-7,5 is optimal for nutrient availability, supporting the life of decomposing bacteria. At pH below 6,0, nutrient availability decreases, while pH above 8,0 increases the availability of certain elements. The electrical conductivity of biogas effluent is about six dSm^{-1}, providing good conductivity to MFC electrodes.

3.2. Open Circuit Voltage Measurement

Microbial Fuel Cell (MFC) is a device that converts chemical energy into electrical energy through the catalytic activity of microorganisms [15]. This bioelectrochemical system uses bacteria to convert organic matter into electrical energy [16]. MFCs can be made in various configurations, including one-chamber and two-chamber systems with KMnO$_4$ or K$_2$Cr$_2$O$_7$ electrolytes. One-room systems have the anode and cathode in the same space without a barrier, while two-room systems separate the anode and cathode with a salt bridge. The salt bridge maintains electrical charge neutrality, allowing the flow of electrons and ions, ensuring spontaneous redox reactions at the electrodes, resulting in a continuous electric current.

In this study, the open circuit voltage of the MFC system was measured for 20 hours to ensure the achievement of a steady state. Measurements were taken periodically every 2 hours to evaluate the changes in voltage over time. Figure 2(a) shows that the one-cell MFC system experienced an increase in voltage until it reached a steady state of about 310,6 mV. Meanwhile, the two-chamber MFC system with KMnO$_4$ and K$_2$Cr$_2$O$_7$ electrolytes reached a steady state at 312,3 mV and 357,1 mV, respectively. These results indicate that each MFC system can achieve stability in open circuit voltage after a certain time.
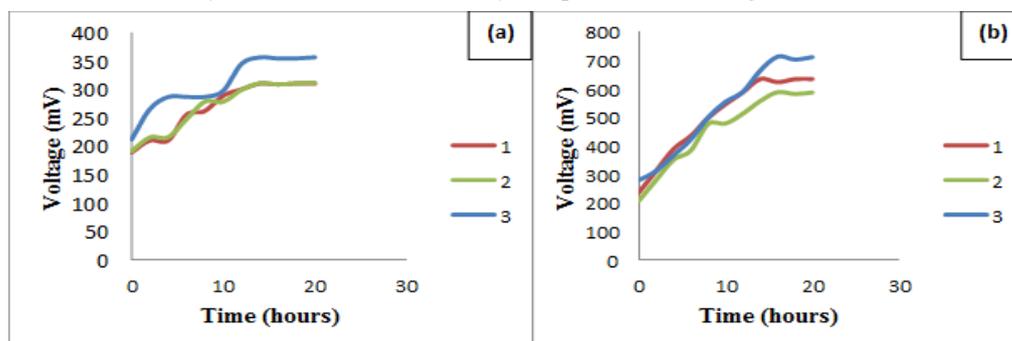
Description:
1. Single-chamber MFC system 2. Two-chamber MFC system using KMnO$_4$ electrolyte 3. Two-chamber MFC system using K$_2$Cr$_2$O$_7$ electrolyte

Figure 2. Comparison of time and voltage (open circuits voltage) generated by one-cell MFC system (a) and seven-cell and ten-cell MFC systems (b).

Figure 2 (b) shows that the open circuit voltage increases to 20 hours. In a one-room MFC system for ten cells, the open circuit voltage occurs in a steady state (fixed condition) at 634,1 mV. In a two-room MFC system using $KMnO_4$ electrolyte for seven cells, open circuit voltage occurs steady state (fixed condition) at 586,7 mV, while using $K_2Cr_2O_7$ electrolyte for ten cells open circuit voltage occurs steady state (fixed condition) at 711,4 mV In. The magnitude of the resulting voltage value is in line with the statement of Tiara et al. [17] that, in general, microbial activity has a peak point until the 18th hour. Microbial activity at the 18th hour is considered a log phase where microbes divide rapidly and constantly follow a logarithmic curve. The speed of growth in this phase is strongly influenced by the medium in which it grows, such as pH and nutrient content, as well as environmental conditions, including temperature and humidity. Microbes require more energy in this phase than in other phases [18].

3.3. MFC System Voltage and Current Measurements

The measurement of electrical energy in this study was carried out in parallel. A parallel circuit is an electrical circuit that can be installed between components in a row. According to Sudarman et al. [5], who took measurements with a parallel circuit, was able to increase the voltage value high enough but did not produce an electric current. The parallel circuit is expected to produce a large enough voltage and an electric current.

Based on the measurement results for the one-cell MFC system (Figure 3a.), the greatest voltage produced is from the dual chamber MFC system using $K_2Cr_2O_7$ electrolyte 412,1 mV. The measurement results of the voltage generated by one chamber MFC is 367,5 mV, and for the two-chamber MFC system using $KMnO_4$, it is 371,2 mV. The one-room MFC system assembled into ten cells (Figure 3b.) had a maximum voltage of 1302,2 mV, and no electric current was obtained. Measurement of two-chamber MFC using $KMnO_4$ electrolyte, which is assembled into seven cells, has a maximum voltage of 1084.3 mV and can produce an electric current of 0,01 mA, while measurement of MFC voltage using $K_2Cr_2O_7$ electrolyte produces a maximum voltage of 1675,8 mV and an electric current of 0,99 mA. The measurement results show that the highest voltage and current are produced in the two-chamber MFC system using the $K_2Cr_2O_7$ electrolyte.

In microbial metabolism, electrons (e-) and protons (H+) are generated, resulting in a potential difference. Electrons move from the anode to the cathode, and H+ balances the difference through salt bridges. At the cathode, H+ and e- react with Cr(VI) metal ions to form Cr(III), which is called a reduction reaction. $K_2Cr_2O_7$, as a strong oxidizer, allows protons from the anode to be rapidly reduced to form H_2O, keeping the potential difference stable in the MFC system, according to the research of Xia et al. [19].
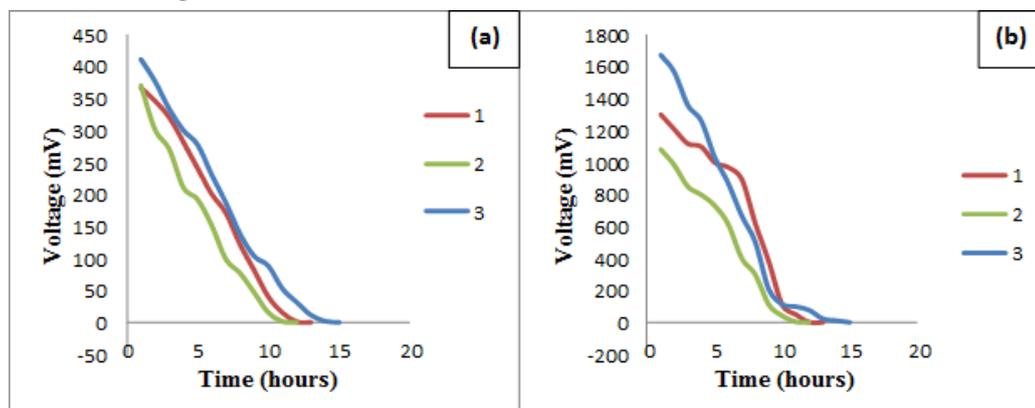
Description:
1. Single-chamber MFC system 2. Two-chamber MFC system using $KMnO_4$ electrolyte 3. Two-chamber MFC system using $K_2Cr_2O_7$ electrolyte
Figure 3. Comparison of time and voltage produced by one-cell MFC system (a) and seven-cell and ten-cell MFC systems (b).

The movement of the voltage measurement graph on the one-cell MFC system (Figure 3a.) and the ten-cell and seven-cell MFC systems (Figure 3b.) experienced a decrease in voltage from the first day of measurement to the last day of measurement. This is probably because the nutrients in the substrate have decreased, and there is no substrate addition, so this impacts microbial growth, which decreases. According to Goel [20], the amount of electrical energy produced by the MFC system can be influenced by microbial activity that utilizes the nutrients contained in the substrate. The more active the microbes are in breaking down organic matter in the substrate, the more free electrons will be produced in the anode chamber. The difference in the number of electrons produced between the anode and cathode chamber causes a potential difference reaction between the two, which a multimeter can detect.

In general, the results of this study have produced voltages that are relatively similar to commercial batteries but still have low amperage. Therefore, to be able to answer this challenge, a circuit is needed that can store energy from MFCs and run electronics at low potential (<500 mV) [21] [22]. This has been done by Lv et al. [23], who used a capacitor to store energy from microbial fuel cells and run sensors using the energy stored in the capacitor. Donovan et al. [21] have also tried to overcome these challenges by using a circuit design called Power Management System (PMS) to run a wireless sensor. The PMS design operates at low potential input regardless of the power generated by the SMFC. PMS starts running the wireless sensor when the SMFC potential reaches 320 mV and keeps running until the SMFC potential drops below 52 mV.

3.4. Characteristics of MFC Bacteria

Bacterial characterization research in biogas effluent using a numerical-phenetic approach. Phenotypic tests, gram painting, and motility tests were used. Identifying isolates aims to reveal specific traits of isolates, which is important for establishing taxonomic status. Four bacterial isolates with different characteristics were found, as noted in Table 2.

Biochemical characteristics of MFC bacterial motility testing showed that the bacterial isolates obtained were mostly nonmotile, classified as bacteria that cannot move or do not have flagella as a means of movement. Bacterial isolates that do not have a means of motion are isolates B_1, B_2, and B_4, while motile isolates with a means of motion are isolates B_3.

Table 2: Biochemical characteristics of MFC bacterial isolates

No.	Isolate	Salt Coloring	Motility Testing	Morphology Test		Description
				Cell Shape	Colony Shape	
1.	B_1	+	Nonmotil	Rod	Circular	Methanobacterium
2.	B_2	+	Nonmotil	Sarcina	Irregular	Methanosarcima
3.	B_3	-	Motil	Spirillum	Irregular	Methanospirillum
4.	B_4	+	Nonmotil	Rod	Circular	Methanobacterium

Gram staining is used to identify bacterial morphology and distinguish between Gram positive and Gram negative bacteria. Table 2 shows that isolates B_1, B_2, and B_4 from biogas effluent are Gram positive bacteria. Gram positive bacteria have cell walls that appear blue/purple under the microscope, due to lower lipid content and thicker peptidoglycan than Gram negative bacteria [24].
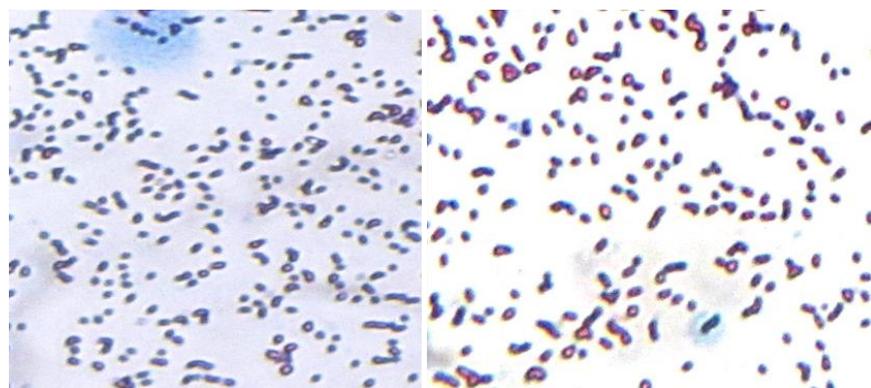
Figure 4. Appearance of bacteria under a microscope

From the specific properties presented in Table 2, it can be seen that the three isolates B_1 and B_4 are gram-positive bacteria with rod shape, B_2 is gram-positive with sarcina shape, and B_3 is gram-negative bacteria with spirillum shape. According to Bergey's Manual of Determinative Bacteriology, the characterisation test results in Table 2 can determine the genus of the bacterial isolates, namely isolates B_1 and B_4 are members of the genus Methanobacterium. Bacterial isolate B_2 is a member of the genus Methanosarcina, and B_3 belongs to the genus Methanospirillum.

Methanogen bacteria belong to one class of Archaebacteria in addition to halophilic and thermophilic by the group Archaebacteria are microorganisms that can survive in extreme areas such as waters with high salt content (halophil). Methanogenic bacteria are obligate anaerobes divided into three groups. Group I includes Methanobacterium and Methanobrevibacter, Group II includes Methanococcus, and Group III includes Methanospirillum and Methanosarcina genera. All are present in anaerobic freshwater environments such as sediments and in the digestive tract of animals such as cattle, sheep, and goats [25].

4. Conclusion

The seven-cell and ten-cell MFC systems, using $K_2Cr_2O_7$ solution, produced a maximum electrical energy of 1675,8 mV and an electrical current of 0,99 mA. After 20 days, the biogas effluent underwent changes with a decrease in organic matter and nitrogen content, resulting in a C/N ratio of 1,19. The effluent pH increased on average to 7,67 (pH H_2O) and 7,54 (pH KCl), with an electrical conductivity of 5,01 dSm^{-1}. The research demonstrates the potential of MFCs as a renewable energy source, a contribution to energy diversification, and the benefits of efficient waste management.

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