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Potential of Rubber Wastewater and Paddy Field Mud as Bioelectricity Producers Using Single-Chamber Microbial Fuel Cell

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ABSTRACT

Rubber wastewater contains many organic compounds that can pollute the environment if directly released. Likewise, paddy field mud also contains many organic compounds that can be utilized with rubber wastewater as an electricity producer through the process of breaking down organic compounds by microorganisms to produce electrons using the microbial fuel cell method. Microbial fuel cell will generate electricity while reducing waste load. This study was conducted to determine the potential of rubber wastewater and paddy field mud in the production of electricity through the process of degradation of organic matter by microorganisms. The treatment was done by combining rubber wastewater and paddy field mud with the ratio of 1:1, 1:2, and 1:3. The results showed that the highest voltage production by rubber wastewater was 228.1 mV and by paddy field mud was 27.4 mV. In the treatment, the highest voltage was 75.3 mV at a ratio of 1:1. There was also a decrease in waste load in the form of COD, BOD, TAN, TSS, and pH. The isolation results on the MFC anode found 6 isolates, namely *Citrobacter* sp.1, *Citrobacter* sp.2, *Citrobacter* sp.3, *Citrobacter* sp.4, *Clostridium* sp., and *Bacillus* sp. It can be concluded in this research that rubber wastewater and paddy field mud have the potential to produce electrical energy through microbial fuel cell. It is hoped that optimization in further research on electricity production will be useful as an environmentally friendly renewable energy.

Keywords: Microbial Fuel Cell, Wastewater, Renewable Energy, Eco-Friendly, Anaerobic Microorganism.

INTRODUCTION

Rubber industry produces quite a lot of wastewater because in its processing, used of water as much as 25-40 m^3 / ton of dry rubber. If not managed properly, this rubber wastewater can cause environmental pollution because this waste still contains many organic compounds with high concentrations [1]. Some rubber industries produce effluent with BOD levels of around 2840 mg/L and COD of 1043 mg/L which do not meet the standard requirements to be released directly into the environment [2]. The high levels of BOD and COD in rubber wastewater will result in environmental damage and the death of aquatic biota. Therefore, further management is needed to handle rubber wastewater.

Environmental damage is not only caused by industrial waste, the use of fossil fuels as a power plant can also pollute the environment. Steam Power Plants (PLTU) produce CO_2, NOx, and SO_2 gases as well as radioactive pollutants that become pollutants in the air [3]. The high pollution generated by the power generation process is in line with the high electricity consumption.

Microbial Fuel Cell will produce energy from the metabolic process of microorganisms [4]. The production of alternative electrical energy by MFC is considered easy to apply, and efficient because the process is safe, does not produce toxic compounds, and uses the latest combination techniques. In recent years, MFC has shown the potential to convert chemical and organic materials into electrical energy while improving the quality of the waste [5].

In this research wants to describe the potential of rubber wastewater and paddy field mud in producing electrical energy through Microbial Fuel Cell (MFC) and the characteristics of bacteria isolated from the anode in Microbial Fuel Cell (MFC).

MATERIAL AND METHODS

This research method uses a survey method. The sampling techniques use a purposive sampling method. The microbial fuel cell consists of an anode in the form of graphite carbon and a cathode in the form of zinc connected using wires and resistors with a resistance of 850Ω. rubber wastewater and paddy field mud are put into 800 mL each. The treatment is given by mixing rubber wastewater and paddy field mud with a ratio of 1:1, 1:2, and 1:3. Then the voltage was measured for 7 days using a digital multimeter.

Bacteria on the MFC anode were isolated by inserting the electrodes that had been used during the bioelectricity production process into Alkaline Peptone Agar (APW) liquid media. Then incubated for 48 hours under dark anaerobic conditions using an anaerobic jar [6]. The growing bacteria were taken as much as 1 mL of suspension with a micropipette and diluted 10-6 using 0.85% physiological NaCl solution and then vortexed to make it homogeneous [7] then inoculated as much as 0.1 mL with the spread plate method on APW solid media and incubated for 48 hours under anaerobic conditions. The bacterial isolate that appears is then purified in order to get a single isolate. The isolates obtained were then observed for their characteristics in the form of macroscopic, microscopic, gram staining, and physiological observations through biochemical tests.

EXPERIMENTS AND ANALYSIS

Electrical Energy Production Through Rubber Wastewater and Paddy Field Mud

Figure 1. shows that the MFC electrical voltage production using rubber wastewater on the first day was 110.6 mV and experienced increases and decreases but were not very significant. On the fourth and fifth days there was a drastic increase until it reached 228.1 mV on the sixth day. Then the electric voltage on the seventh day decreased to 190 mV. In the first three days, the microorganisms adapted to the dark and anaerobic conditions of the MFC chamber so that only certain microorganisms could survive and their activity was not yet stable so that the electrical

voltage produced fluctuated. In line with previous research, bacteria need 2-3 days to adapt to a new environment so that they can use the nutrients around them optimally [8].

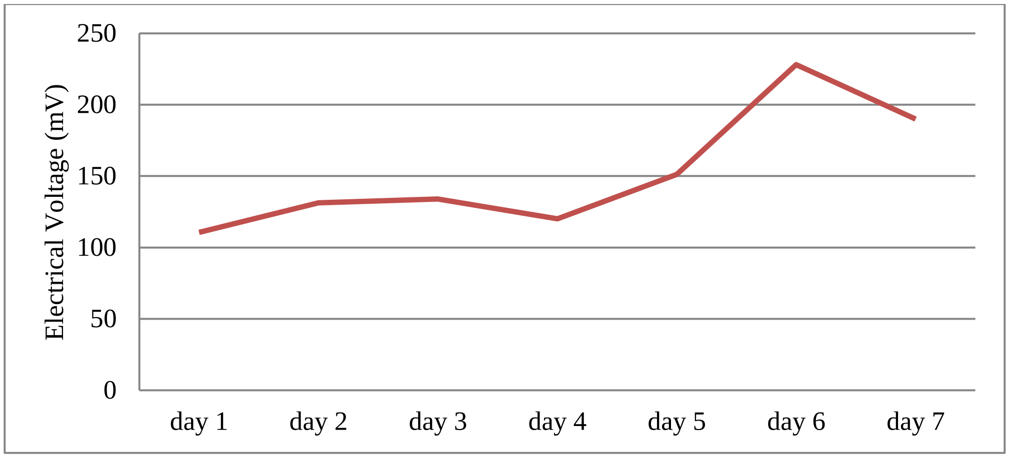


Fig 1: Production of electric voltage through MFC using rubber wastewater

On the fourth to sixth day, the microorganisms have adapted so that bacterial cell growth increases, bacterial metabolism increases, and their ability to break down the substrate also increases so that the electrical voltage produced is quite large. On the seventh day, the decrease in electrical voltage was due to the organic compounds available not being as much as the previous days. This is due to the high metabolic activity of microorganisms that use organic compounds on the previous day so that the availability of organic compounds on the seventh day has decreased a lot. Lack of nutrition or the accumulation of bacterial metabolism products can be toxic to microorganisms resulting in a decrease in metabolic activity and even death [9].

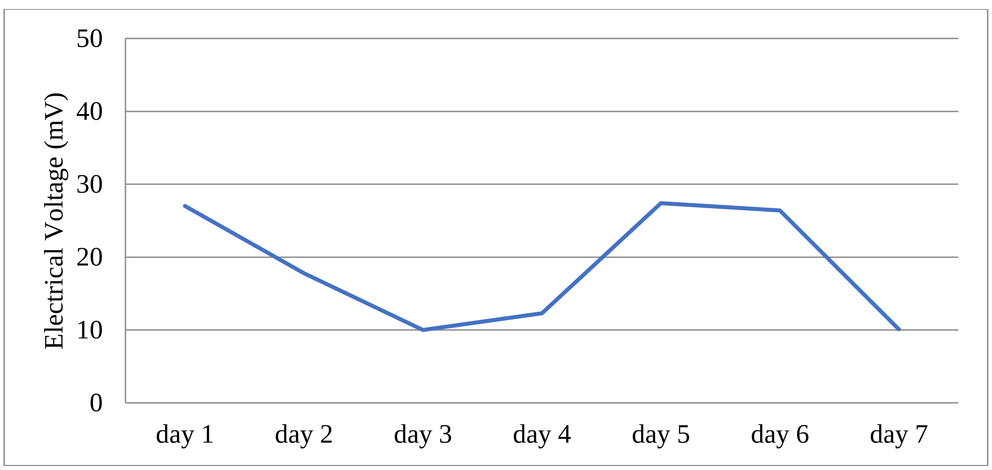


Fig 2: Production of electric voltage through MFC using paddy field mud

Figure 2. shows that the MFC electrical voltage production using paddy field mud on the first day was 27 mV. The low electrical voltage generated on the paddy field mud substrate can be caused by the electrical conductivity of the paddy field mud, thus affecting the output in the form of the electrical voltage produced. The electrical conductivity of a substrate indicates the ionic strength of that substrate. The greater the ionic strength of the substrate, the faster the oxidation and reduction processes that occur in the MFC [10].

On the second and third days it was observed that the electrical voltage decreased. The decrease in electrical voltage on the second and third days is caused by the microorganisms having produced a lot of energy through the breakdown of the substrate to adapt on the first day so that the microorganisms have really adapted on the following day and are not as active as during the adaptation phase. This is in line with previous research, that electrical voltage increased rapidly in the first 15 hours due to high activity of microorganisms during the adaptation period and then decreased in the following hour [11]. On the fourth and fifth days there was an increase in electrical voltage and the highest voltage reached 27.4 mV. This can be caused by microorganisms are grow rapidly so that the number of cells increases at maximum speed and influenced the voltage in MFC [12]. On the sixth day the average electrical voltage produced was constant as on the fifth day. Meanwhile, on the seventh day, the electrical voltage produced began to decrease drastically.

Electrical Energy Production Through a Mixture of Rubber Wastewater and Paddy Field Mud

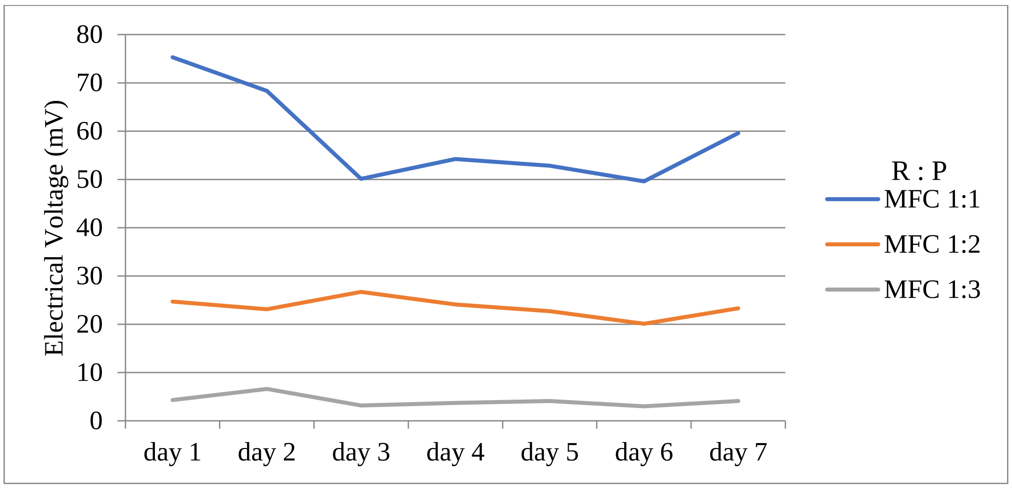


Fig 3: Production of electric voltage using various mixture ratios

Note: R = Rubber wastewater, P = Paddy field mud

In this combination, paddy field mud acts as a sediment and rubber wastewater acts as a substrate. Based on Figure 3. it can be seen that the highest electric voltage is produced in a mixture of rubber wastewater and paddy field mud with a ratio of 1: 1. The highest electric voltage produced is 75.3 mV. At a ratio of 1:2, the highest electrical voltage produced was 26.7, whereas at a ratio of 1:3 the highest electrical voltage produced was only 6.6 mV. This is due to the comparison between the availability of organic compounds and the ability of microbes to be balanced at a ratio of 1:1 so that metabolism is more optimal and the electrical voltage produced is better than other ratios. The combination of substrates in the MFC affects the electrical voltage produced based on the match between the types and volumes when combined. At a ratio of 1:2 and 1:3, the resulting electric voltage is low due to the paddy field mud ratio which has a greater influence on the electron conductivity of the MFC. Paddy mud contains many microorganisms in it so that the formation of biofilm on the anode by bacteria occurs more quickly. This biofilm will thicken as the bacterial cells grow. A thick biofilm will inhibit the capture of electric current, resulting in decrease in electric current.

The process of producing electric voltage obtained from the degradation process of organic compounds by utilizing the metabolism of microorganisms in waste becomes an effective alternative waste processing method using MFC. The decrease in organic matter levels in waste is directly proportional to the electrical voltage produced through MFC, the higher the degradation activity by microorganisms, the higher the percentage of decrease in organic levels in waste [13]. Waste degradation by bacteria in MFC can be seen in Table 1.

Table 1: Results of measurements of rubber wastewater load

Parameter	Before Treatment	After Treatment
COD	146,10 ppm	110,20 ppm
BOD	113,49 ppm	86,74 ppm
TAN	18,50 ppm	15,50 ppm
TSS	180,26 ppm	100,70 ppm
pH	8.2	8.1

Note: COD = Chemical Oxygen Demand, TSS = Total Solid Suspended, pH = Potential of Hydrogen, BOD = Biological Oxygen Demand, TAN = Total Ammonium Nitrogen

The decrease in waste load in the form of COD, BOD, TAN, TSS, and pH levels indicates that microorganisms have degraded the compounds contained in the waste through their metabolic processes and the reduction and oxidation processes occur. Microorganisms grow and metabolize organic matter around their environment as nutrients [14].

Isolation of Microorganisms at the Anode of the MFC

Microorganisms on the anode of the MFC with a substrate of rubber wastewater and paddy field mud with a ratio of 1:1 found bacterial colonies of 5.7 x 108 CFU/mL and on the rubber wastewater MFC found bacterial colonies of 61 x 106 CFU/mL. The existence of this colony indicates that the bacteria found in paddy field mud and rubber wastewater have formed a biofilm on the anode and the metabolic activity of the bacteria is running well so that an electric voltage is produced. The presence of bacterial colonies formed shows that the electricity production process in MFCs is influenced by the ability of these bacteria as electron conductors through their cell metabolism which is exoelectrogenic [15]. The results of the bacterial isolation process from the anode were 6 isolates, in the form of MFC 1, MFC 2, MFC 3, MFC 4, MFC 5, and MFC 6 which were then identified based on their characteristics.

Identification of Bacterial Isolates Based on Several Characteristics

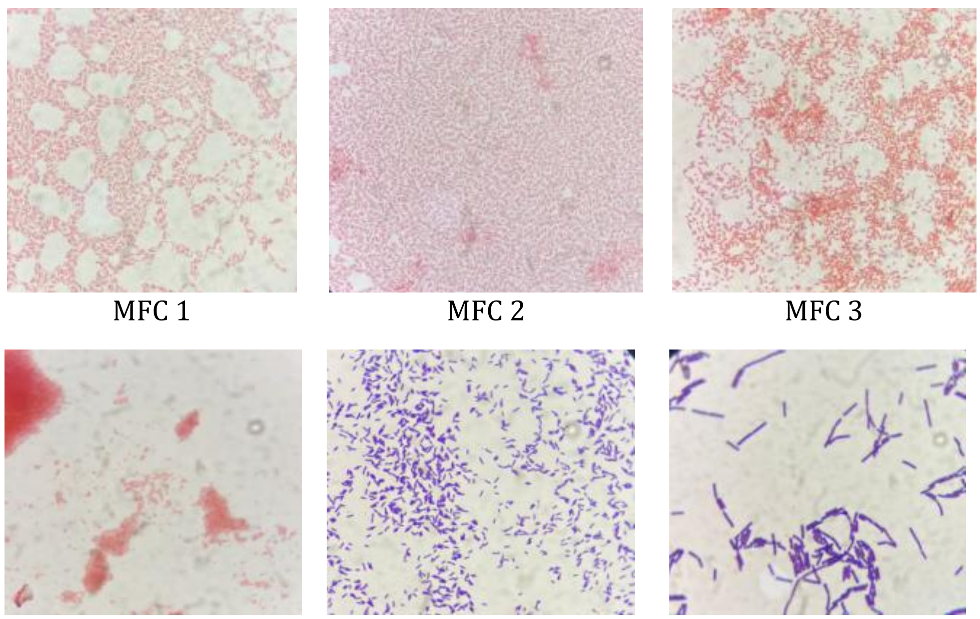
Identification of bacterial isolates was carried out using macroscopic, microscopic characterization stages (Table 2.) and biochemical tests (Table 3.) on the six isolates.

Table 2: Results of morphological characterization of bacterial isolates

Morphological Character	Isolates					
	MFC 1	MFC 2	MFC 3	MFC 4	MFC 5	MFC 6
Macroscopic						
Shape	Round	Round	Round	Round	Widen	Round
Size	Large	Small	Large	Large	Large	Small
Colour	Yellowish	Yellowish	Yellowish	Yellowish	Yellowish	Yellowish
Elevation	Raised	Raised	Raised	Flat	Raised	Flat

Surface character	Shiny	Frown	Shiny	Shiny	Frown	Shiny
Microscopic						
Cell shape	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Cell size	Short	Elongated	Short	Short	Short	Elongated
Morphological Character	**Isolates**					
	MFC 1	**MFC 2**	**MFC 3**	**MFC 4**	**MFC 5**	**MFC 6**
Gram	Negative	Negative	Negative	Negative	Positive	Positive
Endospores	-	-	-	-		Positive
Biochemical Test	(Table 3)					

In Table 2 can be observed that isolates MFC 1, MFC 2, MFC 3, and MFC 4 include bacteria from the gram negative bacilli group. Isolates MFC 5 and MFC 6 belong to the group of gram-positive bacilli. Gram staining of bacterial isolates is presented in Figure 4. and endospores staining presented in Figure 5.



MFC 4 MFC 5 MFC 6

Fig 4: Results of gram staining of bacterial isolates

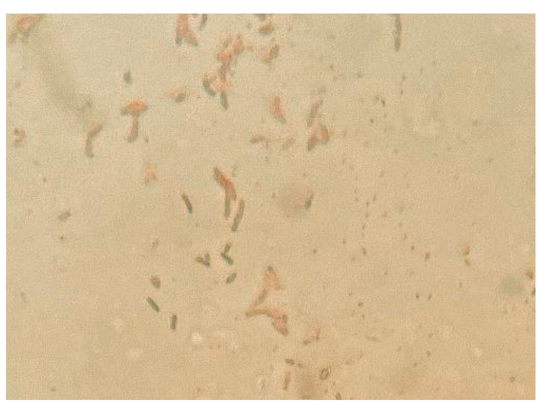


Fig 5: Results of endospore staining of bacterial isolates

In Figure 5. endospores found at MFC 6 isolate. It can be seen that the endospore is characterized by the green color of malachite green while the other cells absorb the reddish color of safranin. Endospores types based on the colorization and observation on MFC 6 isolates type is terminal, which is located at the ends of bacterial cells. Spores or endospores begin to form in the logarithmic growth phase, which only occurs in bacterial cells.

Table 3: Biochemical test results of bacterial isolates

Biochemical Test	Isolate					
	MFC 1	**MFC 2**	**MFC 3**	**MFC 4**	**MFC 5**	**MFC 6**
Gram	-	-	-	-	+	+
Aerob / Anaerob	An	An	An	An	An	An
TSIA	k/k	k/k	k/k	k/k	k/k	k/k
Gas	+	-	+	+	+	+
H2S	-	-	-	-	-	-
Catalase	-	+	+	+	-	-
Oxydase	-	-	-	-	-	-
Motilitas	+	+	+	+	+	+
Indol	+	-	-	-	-	-
Urea	-	-	-	-	-	-
Citrat	-	-	+	+	+	+
Laktosa	+	+	+	+	+	+
Glukosa	+	+	+	+	+	+
Sukrosa	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
MR	+	-	-	-	+	-
VP	-	-	-	-	+	-
O	+	+	+	+	+	+
F	+	+	+	+	+	+
KCN	+	+	+	+		
Arginine	-	-	-	-		
Lysine	-	-	-	-		
Ornithin	-	-	-	-		
Phenylalanine	-	-	-	-		
Aesculin	+	+	+	+		
Arabinose	+	+	+	+		
Raffinose	+	+	+	+		
Sarbitol	+	+	+	+		
Trehalase	+	+	+	+		
Xylose	+	+	+	+		
Dulcitol	+	D	D	-		
Malonat broth	D	D	D	-		
Nitrat					+	+
Gelatin	-	-	-	-	+	+

Note: An = Anaerob, k/k =Yellow/Yellow, +=Positive, - = Negative, D = Dubius (+/-), MFC 1 = *Citrobacter spp*., MFC 2 = *Citrobacter spp*., MFC 3 = *Citrobacter spp*., MFC 4 = *Citrobacter spp*., MFC 5 = *Clostridium sp*., MFC 6 = *Bacillus sp*.

Based on morphological and physiological characterization, the identification stages were carried out by referring to the Manual for The Identification of Medical Bacteria, Second Edition. Based on the identification results, it was found that the bacterial isolates MFC 1, MFC 2, MFC 3, and MFC 4 were bacteria from the Enterobacteriaceae family, namely *Citrobacter* spp. *Citrobacter* spp. is a gram-negative bacterium in the form of a bacillus, is facultative anaerobic, and is commonly found in soil, mud and water. These bacteria are capable of fermenting glucose and other carbohydrates and are also motile. *Citrobacter* spp. is negative for urease and lysine activity [16].

MFC 5 isolate is a bacterium from the Lachnospiraceae family, namely *Clostridium* spp. *Clostridium* spp. is a gram-positive bacterium in the form of a bacillus and can live in anaerobic conditions. These bacteria are distributed in soil, water, animal digestive organs, and other biotopes. (Guo *et al*., 2020). Results of glucose, lactose and sucrose tests on *Clostridium* spp. positive which indicates a biochemical reaction in the bacteria *Clostridium* spp. ferments glucose, lactose, and sucrose to form acid and gas [17].

MFC 6 isolate is a bacterium from the Bacillaceae family, namely *Bacillus* spp. *Bacillus spp*. including a group of gram-positive bacteria that are bacilli-shaped and have flagella [18]. These bacteria are aerobic but some can live in facultative anaerobic conditions [19]. Based on test results, *Bacillus* spp. known to be positive for the citrate test, which indicates that this bacteria has the ability to use citrate as a source of carbon and energy.

CONCLUSION

Based on the results of the research conducted, it can be concluded that rubber wastewater has the potential to produce electricity through a Single Chamber Microbial Fuel Cell with the highest electrical voltage obtained on the 6th day with an average voltage reaching 228.1 mV. Paddy field mud has the potential to produce electricity through a Microbial Fuel Cell Single Chamber with the highest electrical voltage produced on day 5 with an average voltage reaching 27.4 mV. The highest electrical voltage in the combination of rubber wastewater and paddy field mud was in a ratio of 1:1 on the first day of 75.3 mV with a ratio of 1:1 being more potential than other ratios. There were 6 bacterial isolates found on the MFC anode, namely MFC 1, MFC 2, MFC 3, MFC 4, MFC 5, and MFC 6. The identification results showed that 4 isolates were gram negative bacteria, namely *Citrobacter* spp., 1 isolate was gram positive bacilli namely *Clostridium* spp., and 1 isolate was a gram positive bacilli namely *Bacillus* spp.

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