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The Effect of Septage Sludge and Oxidizing Agents in the Microbial Fuel Cells Generating Electricity

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ABSTRACT: Earlier research demonstrated the efficacy of microbial fuel cells in both wastewater treatment and renewable electric current generation. In this process, microbial fuel cells harness the potential of wastewater as a substrate and energy source, enabling microorganisms to generate electric current. Introducing microorganisms sourced from septage sludge acts as a microbial catalyst. Additionally, tofu wastewater is employed as a nutritional resource to support the growth of these microorganisms. A dual-chamber reactor was utilized to carry out this study, featuring an anode and a cathode connected through a salt bridge. Various substrate variations were performed on the anode, specifically with a combination of tofu liquid waste and septage sludge at ratios of 1:1, 1:2, and 1:3. Additionally, different electrolyte solutions, such as $KMnO_4$ and $K_3(Fe(CN)_6)$, were used at the cathode. Using different electrolyte solutions as electron acceptors can enhance the electric current production generated. The study spanned 240 hours of operation, during which electric current, voltage, COD, and BOD measurements were taken at 48-hour intervals. The findings revealed that including septage sludge in a 1:3 ratio yielded the highest current strength compared to other substrate variations, measuring 16.34 mA. When using a 0.25 M $KMnO_4$ as an electrolyte solution, the voltage recorded was 8.78 V. Additionally, the most effective removal of COD and BOD content was achieved with a substrate ratio of 1:3 in the presence of $KMnO_4$, achieving removal rates of 95.12% and 96.45%, respectively. These results indicate that adding septage sludge contributes to increased electricity current production.

KEYWORDS: Electricity; electrolyte; microbial fuel cell; septage sludge; tofu waste water

1. Introduction

The tofu industry is noted to have reached about 84,000 units recently [1]. In Sidoarjo, waste tofu from tofu factories is currently being disposed of without proper treatment. If this waste is not appropriately managed, it can increase organic pollution in water bodies. When tofu

waste is discarded into water bodies, it can cause eutrophication, a process where excessive nutrient levels promote the growth of harmful algae. This can deplete oxygen levels in the water, resulting in the death of aquatic organisms. Chemicals and toxins present in liquid waste can disrupt the balance of ecosystems, leading to the death of fish and other aquatic organisms. Moreover, contaminated water can find its way into underground sources, polluting drinking water supplies and posing a health risk to humans.

To address this issue, the utilization of Microbial Fuel Cells (MFC) has been explored to convert waste into alternative energy sources [2]. MFCs not only have the potential to generate electric current but also possess the ability to concentrate or immobilize heavy metals and remove organic pollutants from wastewater [3]. Microbial fuel cells operate as bioreactors under anaerobic conditions, utilizing microorganisms to catalyze reactions that convert chemical energy from organic substances into electric current [4]. The role of microorganisms is crucial in these fuel cells. Microorganisms may influence the processes of substrate oxidation and oxidant reduction, aiding in the production of energy [3]. Studies have shown that adding microorganisms from septage sludge can significantly enhance power density by up to 116 mW/m^2 [5]. Specifically, septage sludge containing *E. coli* bacteria effectively generates more electrical power in microbial fuel cells [6]. The chemical oxygen demand (COD) for chemical energy in septage sludge has been calculated to be 13 kj/g, or about nine times the energy needed for treatment [7, 8]. Therefore, if the energy present in wastewater could be efficiently recovered, there might be no need for external energy input for running wastewater treatment plants (WWTPs) [8, 9].

In this study, variations of electrolyte solutions were carried out to maximize electric current production. The catholyte solution plays a crucial role in enhancing electricity production as it acts as an electron acceptor in the cathode compartment, which is essential for the operation of microbial fuel cells (MFCs). Potassium permanganate ($KMnO_4$) is commonly used as an electrolyte solution due to its strong oxidizing properties and a standard reduction potential of 1.70 V. By using $KMnO_4$, the potential difference between the anode and cathode is increased, resulting in higher electrical energy production. Tests using the $KMnO_4$ electrolyte solution have shown a significantly greater potential difference of 99.2 mV [10]. The concentration of the electrolyte solution will also affect the amount of power generated [10]. Alternatively, $K_3(Fe(CN)_6$ can be employed as an alternative to potassium permanganate. $K_3(Fe(CN)_6)$ has a lower reduction potential of 0.36 V. One advantage of using $K_3(Fe(CN)_6)$ is that it exhibits a lower standard reduction overpotential compared to carbon electrodes. In a study by [11], using potassium ferricyanide instead of oxygen as the electron acceptor resulted in a higher power density in microbial fuel cells. These findings highlight the importance of selecting an appropriate electrolyte solution, such as $KMnO_4$ or $K_3(Fe(CN)_6)$, as an electron acceptor to optimize the performance and power generation of microbial fuel cells.

2. Materials and Methods

2.1. Materials.

The main substrates used in the study were tofu wastewater obtained from a home industry in Sidoarjo and septage sludge collected from sewage treatment plant (STP) Keputih, Surabaya. Additionally, the following materials were utilized: $KMnO_4$ from MERCK, $K_3(Fe(CN)_6)$

from PUDAK Scientific, KCl from MERCK, Microbial Nutrient Agar from MERCK, NaOH from Merck, HCl, and Aquadest (distilled water). The equipment employed in the study consisted of a microbial fuel cell dual-chamber reactor with a volume of 4 liters, carbon graphite electrode, and copper electrode measuring Ø 10 cm and 100 cm in height. Other equipment used included an Avo-multimeter digital device, a pvc pipe as a "salt bridge", copper cables, cable clamps, and a spectrophotometer.

2.2. Preparation of research.

To assess the levels of Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) in the waste materials, the initial phase of the study involved analyzing the fundamental properties of both tofu waste and septage sludge. Several preparation processes were conducted. Electrode preparation involved submerging copper and graphite electrodes in a 1M HCl (hydrochloric acid) solution for one day, followed by a one-day immersion in a NaOH (sodium hydroxide) solution [12]. This treatment was performed to enhance the electrodes' properties. The electrodes were then rinsed with distilled water to prevent metal contamination and prevent organic substances from adhering to their surfaces [4]. To prepare the salt bridge, a mixture of boiling distilled water, 1M KCl (potassium chloride), and microbial sodium agar was prepared. The ingredients were combined and stirred until a homogeneous and thickened mixture was obtained [13]. The mixture of salt was then inserted into a pipe with a diameter of 1 inch and a length of 10 cm. Both the anode and cathode compartments underwent substrate preparation. Tofu waste and septage sludge were prepared for the anode compartment following the variations specified in Table 1. In the cathode compartment, 0.2M and 0.25M $KMnO_4$, as well as 0.2M and 0.25M $K_3(Fe(CN)_6)$ electrolyte solutions, were prepared by weighing the required amounts and dissolving them in 4000 ml of distilled water. The variations of electrolyte solutions listed in Table 1 were prepared accordingly, considering the specific concentrations and ratios for the experimental conditions.

Table 1. Variation of research.

	Variations of Electrolyte														
	$KMnO_4$							**$K_3(Fe(CN)_6)$**							
Variations															
Molarity of Electrolyte	0,2 M			0,25 M				0,2 M			0,25 M				
Substrate Variations	1:1	1:2	1:3	1:1	1:2	1:3	1:0	1:1	1:2	1:3	1:1	1:2	1:3	1:0	

2.3. Main research.

To construct the dual-chamber reactor, a plastic cube with a volume of 4000 ml, shaped like a rectangle, was placed inside. The salt bridge, in the form of a pipe, was created and connected to the reactor. Copper and graphite electrodes were utilized as the anode and cathode, respectively, in the MFC system. The cathode compartment contained the previously prepared $KMnO_4$ and $K_3(Fe(CN)_6)$ electrolyte solutions, while the anode compartment consisted of the prepared substrates, specifically tofu waste and septage sludge. The anode compartment was maintained under anaerobic conditions to facilitate the desired reactions [14]. To establish electrical connections, power cable clamps and metal cables were used to connect each anode and cathode electrode [15]. A digital multimeter is then attached to the

cable secured by the clamp to measure the electric current and calculate the power density (Figure 1). The power density can be computed using the following formula:

$$Power\ density\ (\frac{mW}{m^2}) = \frac{I\ (mA) \times V\ (volt)}{A\ (m^2)}$$

where I is the electric current, V is the voltage, and A is the surface area of the anode [16].

Before the main research was carried out, the initial substrate analysis was conducted on tofu waste and septage sludge to analyze COD, BOD, VFA, and alkalinity levels. COD was analyzed by the winkler titration method SNI 6989.72:2009, BOD was analyzed by the closed reflux spectrophotometry method SNI 6989.2:2019, and VFA was analyzed by distillation and trimetry, while pH was measured using a pH meter.

Throughout the 240-hour research period, samples were collected every 48 hours to analyze the parameters of COD and BOD. These parameters were important indicators of the system's organic content and pollution load. Additionally, measurements of current strength were taken once every 24 hours to monitor the electrical performance of the microbial fuel cell [17]. The collected data, including the measured current and voltage values and the COD and BOD levels, were further analyzed. A two-way ANOVA statistical analysis was conducted using Minitab 18 software to determine the significance of operating time, the type of electrolyte solution, and the amount of substrate on the electric current, voltage, and decrease in wastewater concentration.

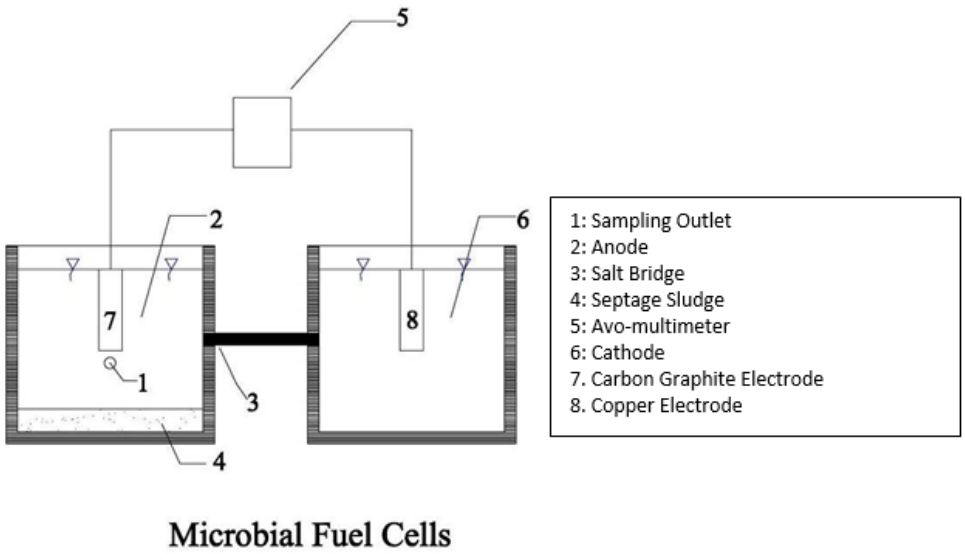

Figure 1. Microbial fuel cell reactor.

3. Results and Discussion

3.1. Characterization of tofu wastewater and septage sludge.

Table 2 presents the original properties of tofu liquid waste and septage sludge. The septage sludge was obtained from the STP Keputih, while the tofu liquid waste was collected from a household enterprise in Sidoarjo. According to Table 2, both wastes exhibit incredibly high levels of organic content, particularly in terms of BOD and COD, which reached 2,345 mg/l

for COD and 778.25 mg/l for BOD, respectively. The organic content, such as COD, BOD, and VFA, was tested in the Institut Teknologi Sepuluh Nopember Water Treatment Laboratory, while the pH of the waste collection site was tested at its location. The initial analysis of the wastewater was conducted to determine the levels of the first pollutants present.

Table 2. Characterization of substrate.

No.	Test Parameter	Result		Unit
		Tofu Wastewater	Septage Sludge	
1	COD	1408	2345	mg/l
2	BOD	587	778.25	mg/l
3	pH	6.4	6.78	-
4	VFA	894,5	1066,44	mg/l

3.2. Production of electric current.

To monitor the voltage and electric current in the microbial fuel cell system, a digital Avo-multimeter was utilized. The multimeter was equipped with clamps and copper wires that were connected to both the anode and cathode electrodes. The positive pole of the multimeter was attached to the cathode, while the negative pole was connected to the anode [18]. The chemical reactions occurring within the anode and cathode compartments resulted in a potential difference between the two ends of the electrodes. This potential difference gave rise to the measured electric current and voltage strengths observed in the system [19]. By measuring these electrical parameters, researchers could assess the performance and efficiency of the microbial fuel cell in generating electric current.

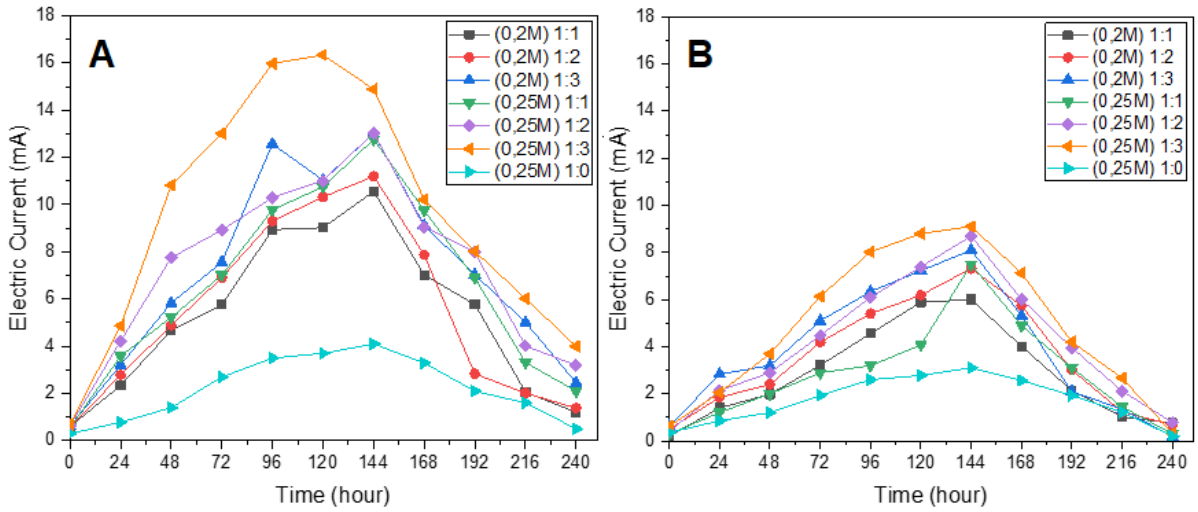

Figure 2. Production of electric current with electrolyte $KMnO_4$ (A) and production of electric current with electrolyte $K_3(Fe(CN)_6)$ (B).

Figure 2 illustrates the trend of electric current throughout the 240-hour experiment. The graph shows that the highest or peak electric current was recorded at time-120 for the variation using $KMnO_4$ as the electrolyte with the substrate ratio of tofu wastewater and septage sludge at 1:3 [20]. Similarly, the maximum or optimal current was observed at time-144 for the variation using $K_3(Fe(CN)_6)$ as the electrolyte with the substrate ratio of 1:3 [20].

The electric current exhibited a trend from the first day up to 144 hours, indicating a relatively stable or increasing current flow. Septage sludge microorganisms oxidize biodegradable molecules, such as acetic acid in tofu wastewater, to produce electrons, protons, and CO_2. The electrons are then transferred to the anode electrode, while the protons move from the anode to the cathode through a salt bridge. These electrons travel through an external circuit to reach the cathode electrode. The interaction between the electrons and protons generates a potential difference, creating an electric current between the cathode and anode, thereby producing electrical power [6]. The reactions contained in this study is summarized as follows:

$$\text{Anode : } C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+ \quad (1)$$
$$\text{Katode : } 6O_2 + 24e^- + 24H^+ \rightarrow 12H_2O \quad (2)$$
$$\text{Overal : } C_6H_{12}O_6 + 6O_2 \rightarrow 6O_2 + 6H_2O + \text{Energy} + \text{Biomass} \quad (3)$$

Protons and electrons coming from the anode are used to reduce Mn^{7+} to Mn^{4+} in MnO_4, besides Fe^{3+} to Fe^{2+} in $Fe(CN)_6^{3-}$ [10]. The reaction that occurs is as follows:

$$MnO_4^- + 4H^+ + O_2 \rightarrow MnO_2 + 2H_2O \qquad E^0 = 1{,}70\ V \quad (4)$$
$$4\ Fe(CN)_6^{4-} + 4H^+ + O_2 \rightarrow 4Fe(CN)_6^{3-} + 2H_2O \qquad E^0 = 0{,}36\ V \quad (5)$$

This trend is attributed to the metabolism of microorganisms in the microbial fuel cell. The metabolic processes of microorganisms generate a more significant number of protons and electrons, leading to the production of more potential electricity. However, after 144 hours, the electric current started to decline. This decline can be attributed to various factors, such as the increased capacity of the reactor, depletion of nutritional or carbon sources, and the presence of inhibitory substances that may interfere with the microorganisms' metabolic activity. This decline in electric current indicates that the microorganisms may have entered the death phase, where their metabolic activity becomes less efficient or ceases altogether [21]. It is worth noting that similar findings have been reported in other studies, suggesting that the decline in electric current over time is a common occurrence [22]. Microorganisms experiencing challenges in decomposing organic material might be indicated by a decrease in an electric current produced [23].

Due to the higher standard reduction potential of $KMnO_4$ compared to $K_3(Fe(CN)_6)$, $KMnO_4$ exhibits higher electrical conductivity and leads to higher electric current and voltage in the microbial fuel cell [10]. $KMnO_4$ has a standard reduction potential of 1.70 V, whereas $K_3(Fe(CN)_6)$ has a standard reduction potential of 0.36 V. A higher standard reduction potential implies a greater impact on the potential of the anode and cathode [10]. This aligns with the study's findings by [24], which suggest that $KMnO_4$ can increase power density by 11 times more than ferricyanide and oxygen. Furthermore, using a higher concentration of $KMnO_4$, such as 0.25 M, results in higher current and voltage compared to using a lower concentration like 0.2 M. Research by [25] supports this observation by stating that increasing the molarity of the electrolyte solution can enhance the electric current and voltage. This is attributed to the increased electron transfer rate when more electron acceptors are available at the electrode, facilitated by a higher concentration of the electrolyte solution.

3.3. Production of voltage.

The voltage measurements presented in Figure 3A show an increasing trend in voltage in accordance with the electric current. This relationship can be explained by Ohm's equation, which states that voltage (V) is equal to the product of current (I) and resistance (R), V = I x R [26]. When the resistance is higher, a smaller electric current is generated. In the initial voltage measurements with a 0.25 M $KMnO_4$ electrolyte solution, the values were 0.657 V for the substrate ratio of 1:1, 0.645 V for the substrate ratio of 1:2, and 0.663 V for the substrate ratio of 1:3. These values then increased from 24 hours to 144 hours, reaching 3.225 V for the ratio of 1:1, 4.319 V for the ratio of 1:2, and 9,302 V for the ratio of 1:3. This increase in voltage can be attributed to the sharp increase in metabolic activity of microorganisms, resulting in increased electricity production through the microbial fuel cell process. However, after reaching the peak, the voltage begins to decline, indicating a limitation in the substrate or nutrient availability. This decrease in voltage can also be influenced by an increase in resistance (R) in the system, as mentioned previously [27]. In line with research conducted by [28], using septic tank waste as a substrate and the addition of B.Subtilis bacterial inoculum as a catalyst, there was a spike in voltage at hour 0 to hour 8 up to 140.7 V then dropped at hour 8 to hour 18 up to 26.90V caused by the growth phase of bacteria.

Figure 3B displays the maximum voltage for each substrate ratio using a 0.25 M $K_3(Fe(CN)_6)$ electrolyte solution. The recorded voltages are 1.198 V for a substrate ratio of 1:1, 1.307 V for a substrate ratio of 1:2, and 1.6 V for a substrate ratio of 1:3. These values represent the highest voltages achieved during the experiment with the specified electrolyte concentration and substrate ratios. It should be noted that the voltage and electric current generated in microbial fuel cells are influenced by the duration of operation time. The longer the operation time, the more opportunity for microbial metabolic activity and electron transfer, which can contribute to higher voltage and electric current production.

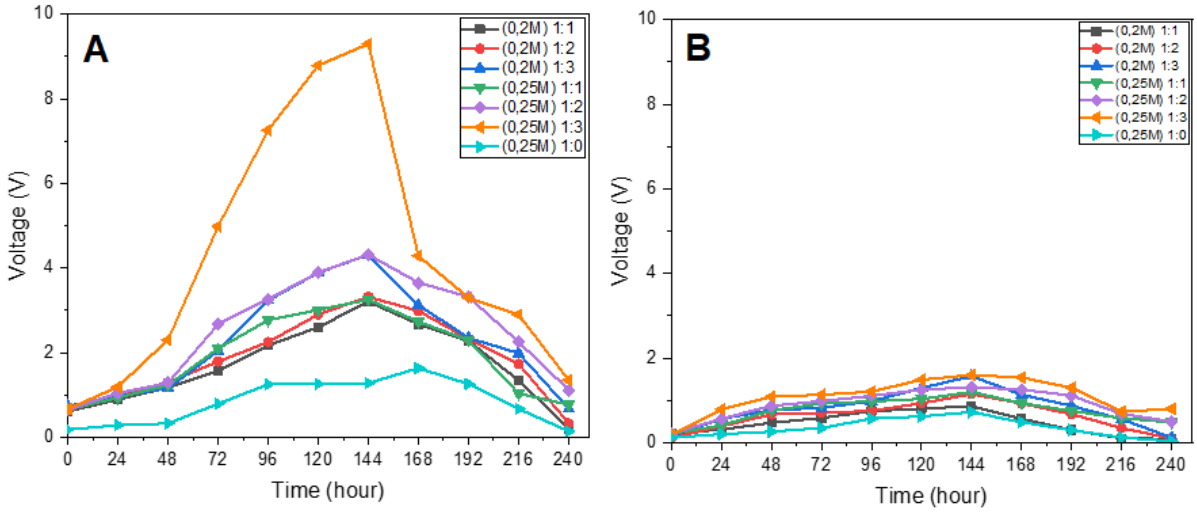

Figure 3. Production of Voltage with Electrolyte $KMnO_4$ (A) and production of voltage with electrolyte $K_3(Fe(CN)_6)$ (B).

The two-way ANOVA test was conducted to find the effect of the research operation time, the type of electrolyte used, as well as variations ratio of substrate. This test has a P-value (0,045) < α (0,05), so H_0 is rejected and H_1 is accepted. This means that variations

electrolyte, variations ratio of substrate affects the magnitude of the current and voltage. The P-value results imply that in the research operation time as well as the type of electrolyte solution used and the variation of substrate comparison have a significant effect at the 0.05 significance level on the strong current and voltage values presented.

3.4. COD Removal

The MFC system's effectiveness in reducing organic pollutants in wastewater was demonstrated through the removal efficiency data presented in Figure 4. Research by [29] indicated that the MFC system produced electric current, voltage, and reduced the organic load in wastewater. Among the various combinations, the $KMnO_4$ 0.25 M with a substrate ratio of 1:3 exhibited the highest COD elimination efficiency at 96.16%. The combination of 0.2 M $KMnO_4$ with a 1:3 ratio achieved a COD elimination efficiency of 90.25%. According to [29], the longer the MFC system operated, the greater the reduced organic content in the wastewater. This was attributed to the interaction between the waste and microorganisms in the anode compartment, which decomposed organic matter [30].

In Figure 4B, for the $K_3(Fe(CN)_6)$ electrolyte solution variations, higher COD reduction efficiency was observed for the substrate ratios of 1:2 and 1:1, while the 1:0 ratio (control) exhibited lower current compared to other comparisons. This suggested that adding septage sludge in combination with $K_3(Fe(CN)_6)$ 0.25 M and a 1:3 substrate ratio resulted in a COD reduction efficiency of up to 80.4%. After the electrons passed through the external circuit and reached the cathode, they combined with the electron acceptor, in this case, the $KMnO_4$ electrolyte solution, to reduce oxygen or other reduced molecules such as nitrate [31]. Microorganisms present in the wastewater oxidized biodegradable molecules like acetic acid, generating protons, CO_2, and electrons. The interaction of organic molecules in the wastewater with $KMnO_4$ and $K_3(Fe(CN)_6)$ as electron acceptors during the cathodic reduction process led to the oxidation of organic matter and a decrease in COD load. The biochemical reactions are at reactions 1, 2, 3, and 4.

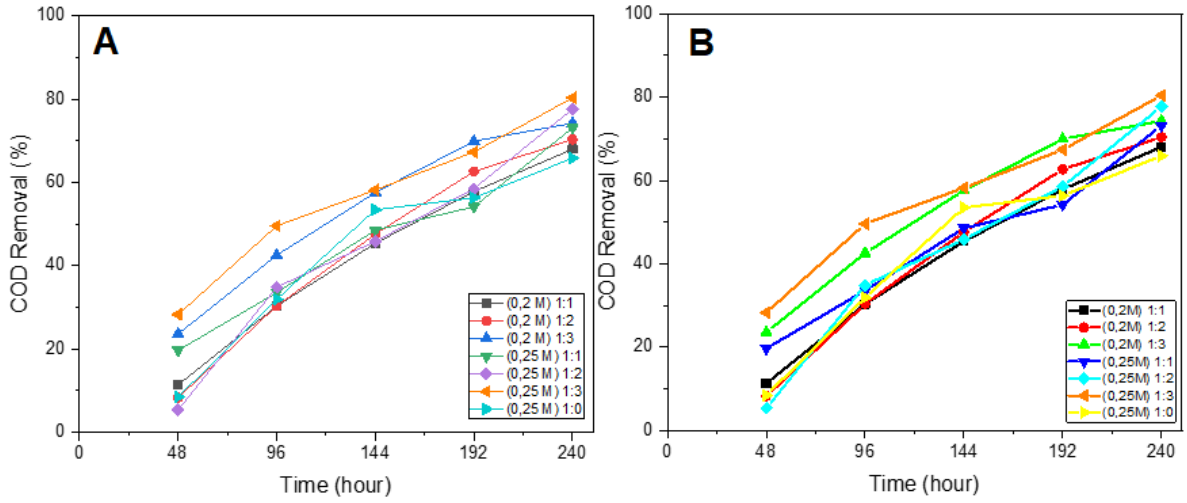

Figure 4. COD removal with electrolyte $KMnO_4$ (A) and COD removal with electrolyte $K_3(Fe(CN)_6)$ (B).

Based on the study's findings, which demonstrated that the substrate combination of liquid tofu waste and septage sludge at a 1:3 ratio exhibited higher COD reduction efficiency than other variations, it can be concluded that the microorganisms present in septage sludge

contributed to the reduction of organic compounds in the waste. The two-way ANOVA test was conducted to find the effect of the research operation time, the type of electrolyte used, as well as variations ratio of substrate. This test has a P-value (0,001) < α (0,05), so H_0 is rejected and H_1 is accepted. This means that variations electrolyte, variations ratio of substrate affects the reduction of COD. Additionally, according to research by [32], adding sludge can enhance electricity generation by 277 mA/m^2. The use of anaerobic sludge as substrates can reduce COD by 90% [28], while in [33] research using pure domestic sludge waste can reduce COD by 93%.

3.5. BOD Removal.

Figure 5 demonstrates the increase in the microbial fuel cell's ability to reduce BOD (Biochemical Oxygen Demand) with increased operating time. The variation using 0.25 M $KMnO_4$ with a substrate ratio of 1:3 exhibited the highest BOD reduction effectiveness at 96.45% among the $KMnO_4$ electrolyte variations. This is consistent with the higher COD reduction effectiveness observed in the same variation using 0.25 M $KMnO_4$ and a substrate ratio of 1:3. Similarly, the $K_3(Fe(CN)_6)$ electrolyte solution with a molarity of 0.25 M and a substrate ratio of 1:3 achieved a BOD reduction efficiency of up to 80.40%, surpassing other variations.

During the first 144 hours, microorganisms undergo an exponential growth phase characterized by rapid cell proliferation [34]. Each cell in the population divides into two cells, resulting in a significant reduction in BOD values due to the metabolic activities of the microbes that consume organic materials in the substrate. Subsequently, between 144 and 192 hours, the system enters a stationary phase where the growth and death rates of bacteria become equal. A death phase follows, during which microorganisms experience a decline in BOD values, indicating a decrease in the number of bacteria. This decline occurs steadily, indicating a stagnant decrease in BOD values.

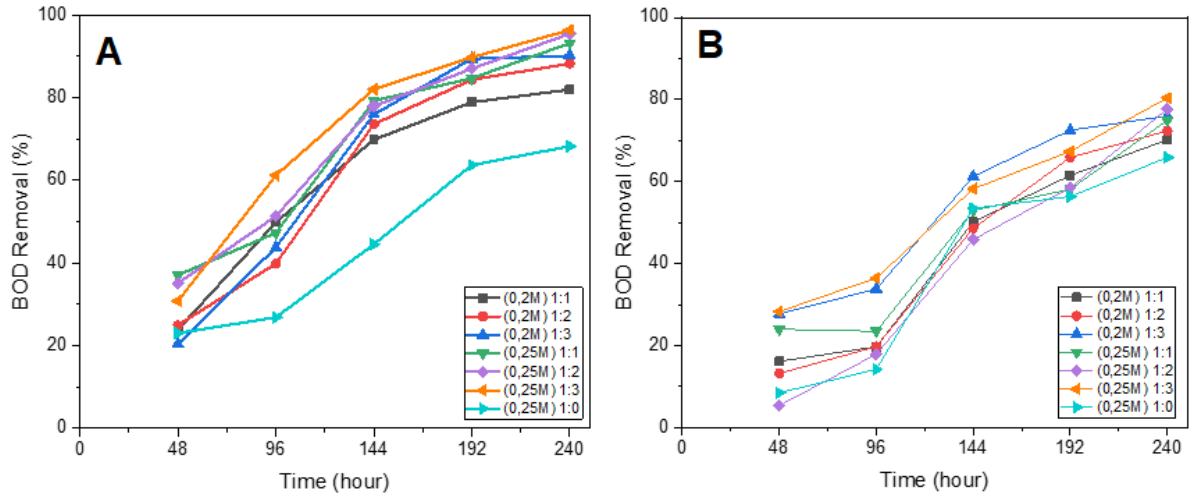

Figure 5. BOD removal with electrolyte $KMnO_4$ (A) and BOD removal with electrolyte $K_3(Fe(CN)_6)$ (B).

According to research [35], during the study, there was a metabolic process of microorganisms in reducing organic matter in wastewater, followed by an increase in electrons and protons. The consumption of oxygen is proportional to the decomposition of organic substances [36]. The addition of sludge can impact the BOD content of wastewater,

as it introduces microorganisms that contribute to the decomposition and reduction of organic matter. The reactions are at reactions 1, 2, 3, and 4. The two-way ANOVA test was conducted to find the effect of the research operation time, the type of electrolyte used, as well as variations in the ratio of substrate. This test yielded a P-value (0.001) < α (0.05), so H_0 is rejected, and H1 is accepted. This means that variations in electrolyte and variations in the ratio of substrate affect the reduction of BOD.

3.5. Anodic electrode investigation of microbial biofilm.

The electrode material characterization test using SEM serves to observe the morphology and biofilm of the microbial bacterial community, as well as possible by-products of bacterial metabolism during the study. Figure 6 shows the results of SEM analysis at 3000x magnification. Figure 6A shows the condition of the carbon graphite electrode before the study, while Figure 6B shows the carbon graphite electrode after the study lasted for 240 hours. The graphite carbon electrode after the study showed the formation of a biofilm on the electrode surface. Microorganisms that form biofilms on the electrode surface affect electron transfer. Biofilms are microbial communities attached to surfaces and consist of extracellular polymeric substances [37]. Microorganisms attach to a suitable surface, form colonies and aggregations, start producing exopolysaccharides, and eventually form a mature biofilm. Occasionally, the biofilm is released, and the microorganisms go back to growing freely to build a new biofilm. The bacteria use their flagella to move freely around in a liquid medium during the free-living phase. The pili are employed for surface adherence as well as for movement and bacterial aggregation [38–40].

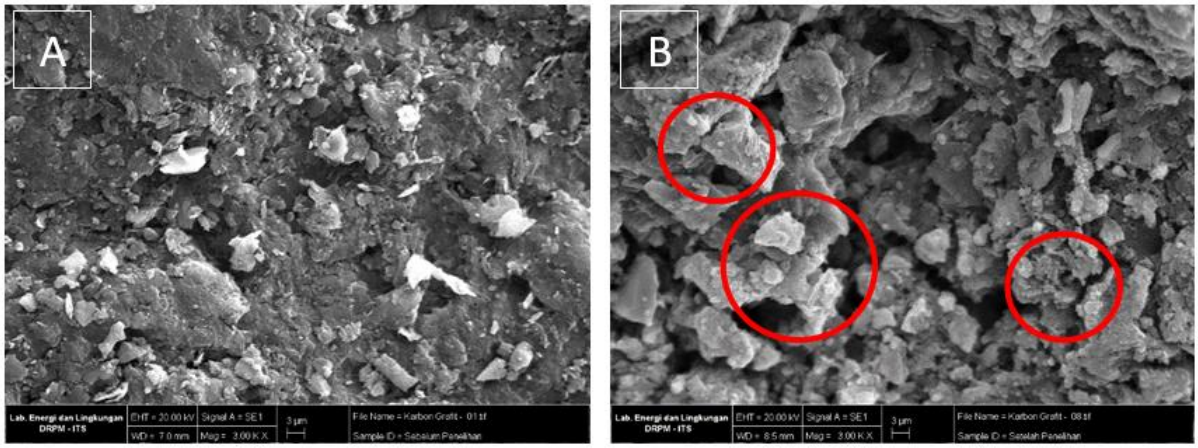

Figure 6. SEM of graphite electrode: before treatment (A) and after treatment (B).

The thickening of the biofilm layer was influenced by the length of operational time. A biofilm that became increasingly attached to the electrode formed a crust (extra resistance) which caused electrons not to be able to enter the layer; this was also called the death phase. This caused the performance of the electrode on the anode to decrease, and the electron transfer process was inhibited, which was characterized by a decrease in electric current [41, 42]. According to the experiment of [43], the biofilm on the electrode surface mostly determined the current output of MFC, which was essential to MFC performance. The growth of biofilm on the electrode caused the deepest layer of biofilm to experience a decrease or even a lack of nutrients, leading to deteriorating conditions [44].

In the research conducted by [33], the effect of the MFC system on tofu liquid waste with a mixture of rice field sludge as an additional microorganism was discussed. With a volume ratio of 1:1, a maximum result of 1.261 mA was obtained at 4 days. The value in this study differed from the study, where the maximum value produced was 16.340 mA using a ratio of 1:3 of tofu liquid waste and septage sludge. The resulting current was 16 times greater when compared to the current generated by the MFC system using additional septage sludge. Another observation of the results of previous research conducted by [45], using tofu liquid waste and $KMnO_4$ electrolyte solution as an electric current generator, gave the highest voltage and current values of 0.78 V and 0.29 mA. The nutrients contained in the substrate were an important factor in producing efficient electricity. Nutrients used ranged from simple organic matter to complex mixtures, such as in wastewater [44].

In the research conducted [10], using $KMnO_4$ electrolyte with molarity variations of 0.1M, 0.15M, 0.2M, and 0.25M, the maximum voltage result was obtained at 99.2 mV at the 26th hour. In $K_3(Fe(CN)_6)$ electrolyte with the same molarity variation, the maximum result was 48.6 V at the 26th hour. This was different from this study, where adding 0.25M molarity variation could increase the voltage result by 9.302 V at the 144^{th} hour. $KMnO_4$ as an electrolyte is a strong oxidizer with a standard reduction potential of 1.70 V and provides a potential difference at the anode and cathode that gets bigger, so that the electrical energy generated also increases. In research, the concentration of the electrolyte solution would have an influence on the amount of electricity generated [10]. $KMnO_4$ had a stronger ability to react with protons and electrons transferred in the anode chamber when compared to dissolved oxygen [43]. This showed that the type of substrate, type of microorganism, type, and amount of molarity of the electrolyte solution could be factors that affected the electrical value of the MFC system [44].

From the SEM analysis, it was found that after the study, there was biofilm present. The challenge of MFC itself was that if the biofilm became increasingly attached to the electrode, it would form a crust (extra resistance) which caused electrons not to be able to enter the layer. Together, the biofilm on the anodic sides may have been responsible for the sudden decrease in power generation that was identified after approximately 240 hours of stable power generation using MFC. In prevention, routine cleaning of the electrodes and the MFC system could help remove the biofilm that had formed. Cleaning could be done using disinfectant solutions or physical methods such as scrubbing or washing [46].

4. Conclusions

The application of 0.25 M $KMnO_4$ in the microbial fuel cell system using a ratio of 1:3 of tofu wastewater and septage sludge resulted in significant achievements. The 1:3 ratio generated the highest current intensity and voltage at 144 hours, reaching 14,890 mA and 9,302 V, respectively. This indicated that the combination of $KMnO_4$ and the 1:3 substrate ratio was highly effective in generating electricity. Septage sludge, rich in organic matter and microorganisms, contributed to the high electron and proton production within the microbial fuel cell reactor. Compared to using $K_3(Fe(CN)_6)$ electrolyte, the use of $KMnO_4$ resulted in increased current and voltage outputs. Furthermore, the 0.25 M $KMnO_4$ variation with a 1:3 substrate ratio exhibited a remarkable power density of 42,000 mW/m² at the 144th hour, indicating a high energy generation capacity. This variation also demonstrated the highest COD reduction efficiency, achieving 96.19% at the 240 hours. These findings highlighted the

effectiveness of using $KMnO_4$ and the 1:3 substrate ratio in the microbial fuel cell system for efficient electricity production and significant reduction of organic pollutants, as indicated by the high COD and BOD reduction efficiencies. Such a combination held promise for wastewater treatment applications, offering a dual benefit of electric current and organic pollutant removal.

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Competing Interest

The authors declare no conflict of interest.

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