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Dual-Chamber Microbial Fuel Cell for Bioelectricity Generation Using Coastal Sediments: A Case from Kendari Bay

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Abstract. This study evaluates the potential of Kendari Bay sediment as an alternative source of electrical energy through the dual-chamber Sediment Microbial Fuel Cell (SMFC) technology. The research focused on sediment characterization, performance analysis using an aerator and $KMnO_4$, post-operation substrate changes, and the identification of electrogenic bacteria. The results showed that the sediment contained 43.24% moisture, 4.23% organic carbon, 1.08% total nitrogen, a C/N ratio of 3.92, pH 7.38, and conductivity of 11.56 mS. The SMFC generated a voltage of 0.404 V (aerator) and 1.628 V ($KMnO_4$), along with a current of 5.0 µA. After SMFC operation, organic content decreased, with 42.65% moisture, 4.06% organic carbon, 0.97% total nitrogen, a C/N ratio of 4.19, pH 7.86, and conductivity of 15.78 mS. Identified bacteria were Gram-positive *Bacillus spp.* These findings demonstrate that aerator and $KMnO_4$ application in dual-chamber SMFC significantly enhance energy conversion efficiency using marine sediment.

Keywords: Dual-Chamber MFC, Bioelectrogenesis, Marine Sediment, *Bacillus spp.*

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1. Introduction

The need for environmentally friendly alternative energy is becoming increasingly urgent amid rising global energy consumption and the significant contribution of greenhouse gas emissions from fossil fuels [1]. A report by the International Energy Agency (IEA) notes that the energy sector still accounts for more than 70% of total global carbon emissions, requiring the development of effective and affordable sustainable energy sources [2]. In Indonesia, the realization of renewable energy in 2023 has only reached around 13.2% of the total national power plant capacity, or around 13.3 GW, with the most significant portion coming from hydropower (8%) and geothermal energy, while solar and wind energy still contribute very little. This figure is still far from the 23% renewable energy mix target by 2025, requiring innovative efforts to accelerate the development of clean, affordable, and sustainable energy sources [3].

One bioenergy technology with development potential is the Sediment Microbial Fuel Cell (SMFC). This bioelectrochemical system utilizes the metabolic activity of electrogenic microorganisms to convert chemical energy from organic materials into electricity [4]. The two-chamber system in SMFC, which separates the anode and cathode chambers, has been proven to improve stability and electron transfer efficiency [5]. In this system, a salt bridge is considered superior to polymer membranes such as Nafion because it is cheaper, easier to manufacture, resistant to the chemical conditions of water, and relatively more resistant to biofouling [6] .

In addition, selecting anodic substrates has a significant effect on SMFC performance. Marine sediments are known to be rich in organic matter and natural electrogenic microbial communities, enabling them to generate bioelectricity without the addition of external carbon [7]. Kendari Bay sediments have estuarine ecosystem characteristics with a mixture of fresh and salt water that carries high nutrient flows, creating a dynamic sediment environment rich in organic matter and supporting the growth of electrogenic microbes [8]. This tremendous potential has not been widely explored, especially in the context of applying a two-chamber SMFC with a salt bridge and cathode treatment using an aerator and $KMnO_4$.

Based on this, this study aims to evaluate the potential of Kendari Bay sediment as a substrate for a two-chamber SMFC based on a salt bridge. The study focused on sediment characterization, system performance testing with different cathode treatments (aerator and $KMnO_4$), and identification of electrogenic bacterial communities. The results of this study are expected to contribute to the development of alternative energy based on bioelectrochemical technology, while supporting sustainable coastal resource management strategies.

2. Methods

2.1. Tools and materials

The tools used in this research include jars, N.Y.A ETERNA cable (1 x 2.5 mm), graphite sheet, water pass hose, digital multimeter, pH meter, conductometer, autoclave, exicator, dark bottle, tongs, measuring flask, Erlenmeyer, measuring cup, UV-Vis spectrophotometer, oven, and measuring pipette. The materials used in this research were Kendari Bay Sediment, distilled water, ferrous ammonium sulfate (p.a.), ferroin indicator (p.a.), NaCl (p.a.), $KMnO_4$ (p.a.), $K_2Cr_2O_7$ (p.a.), C standard solution of glucose, N standard solution of ammonium sulfate, H_2SO_4 (p.a.), NaCl (p.a.), NaCl (p.a.), NH_4Cl (p.a.), KH_2PO_4 (p.a.), $MgSO_4.7H_2O$ (p.a.), $NaHCO_3$ (p.a.) and KCl (p.a.), Sangga Tartrate solution, Na-phenate solution, NaOCl, and phosphate buffer solution pH 7.0.

2.2. Sediment Kendari Bay Sampling

Sediment sampling in Kendari Bay uses the Purposive Random Sampling method, adjusting to the geographical conditions of the bay, which is divided into inner and outer parts. Samples were selected based on the distribution of organic matter entering the bay. Samples were taken using an Ekman grab and then put into a sample box to be analyzed in the laboratory.

2.3. Measurement of C-organic

A 0.5 g sample was placed in an Erlenmeyer and mixed with 5 mL of 1 N $K_2Cr_2O_7$ and 7.5 mL of concentrated H_2SO_4, shaken and allowed to stand for 30 minutes, then diluted to 100 mL. The next day, the absorbance of the clear solution was measured using a UV-Vis spectrophotometer at 561 nm. For comparison, standards of 0, 250, 500 and 1000 ppm C of glucose were made by pipetting 0, 5, 10 and 20 mL of 5000 ppm C standard glucose solution into a 100 mL volumetric flask with the same treatment as the sample work. A standard curve was made, and the sample uptake values were calculated using the curve line equation [1][8].

2.4. Measurement of N-total

A 0.5 g sample was mixed with 1 g selenium and 3 mL concentrated sulfuric acid, then deconstructed at 350 °C for 3-4 hours until white vapour appeared and a clear extract was obtained. After cooling, the extract was diluted with distilled water to 50 mL, shaken homogeneously, and left overnight to precipitate the particles. This extract was used for nitrogen measurement with a spectrophotometer. A sample extract of 2 mL was pipetted into a test tube. For comparison, a standard series of 0, 250, 500 and 1000 ppm N of ammonium sulfate was made by pipetting 0, 5, 10 and 20 mL of 5000 ppm N standard solution of ammonium sulfate into a 100 mL volumetric flask with the same treatment. A 4 mL of Tartrate and Na-phenate buffer solution was added, shaken, and allowed to stand for 10 minutes. Then, 4 mL of 5% NaOCl was shaken, and the absorbance was measured with a spectrophotometer at 636 nm after 10 minutes [1][8].

2.5. Measurement of pH

A 10 g sediment sample was put into a shaker bottle, then 50 mL of distilled water was added. The mixture was shaken using a shaking machine for 30 minutes. Then, the pH was measured using a pH meter calibrated with phosphate buffer solution pH 7.0 [1][8].

2.6. Measurement of Conductivity

A total of 10 g of sample was mixed with 50 mL of distilled water in a shaker bottle and shaken for 30 minutes. Electrical conductivity was measured using a conductometer with a platinum electrode calibrated using NaCl standard solution. The electrode was washed and dried before calibration and measurement [1][9].

2.7. Preparation of Salt Bridge

The salt bridge was prepared by mixing 400 grams of agar, 2 liters of water, and 200 grams of KCl. The mixture was heated until homogeneous, then poured into a prepared tube or pipe and left to solidify [1].

2.8. Manufacture of SMFC Reactor

The MFC reactor consists of two anode and cathode compartments, each with a volume of 500 mL, connected by a salt bridge. The circuit arrangement is shown in the following figure.

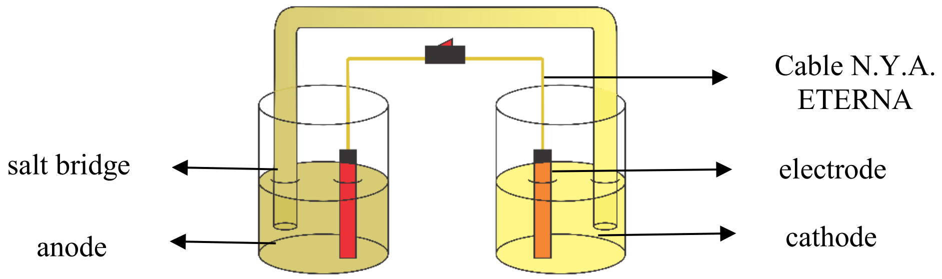


Figure 1. SMFC circuit schematic

Electrical energy in the SMFC system is measured through voltage and current. Initial measurements were taken every 3 hours for 24 hours to determine the maximum open circuit voltage, which affects the energy production of the SMFC. In addition to voltage, current strength was also measured.

2.9. SMFC voltage and current measurements

The anode compartment was filled with sediment containing microbial consortium, while the cathode was filled with $KMnO_4$ electrolyte with an aerator for oxygen. Electrical energy (current and voltage) was measured daily using a digital multimeter to monitor the exponential phase of the microbes. The MFC system was optimized by connecting two cells in parallel to increase the electrical energy. The system resistance was then calculated based on the following equation.

$$\text{Resistance } (\Omega) = \frac{\text{V}}{\text{I}}$$

Then, calculate the power density of the SMFC system (mW/m^2) using the following equation.

$$\text{Power Density}\left(\frac{mW}{m^2}\right) = \frac{\text{I x V}}{A}$$

2.10. Data Analysis.

Data analysis in this study was performed using Analysis of Variance (ANOVA) with the assistance of Jamovi software at a 95% confidence level. The criteria at this confidence level indicate that if the p-value < 0.05, the null hypothesis is rejected, meaning there is a significant difference between treatment groups. Conversely, if the p-value ≥ 0.05, the null hypothesis cannot be rejected, indicating no significant difference between treatment groups.

2.11. Identification and Characterization of MFC Microorganisms

MFC bacteria from Kendari Bay sediments were isolated using the pour plate method on Nutrient Agar (NA) media. The selection of isolates was based on the morphological characteristics of colonies grown on NA media. Phenotypic characterization of the isolates was carried out based on morphological, biochemical, and physiological traits. The observed colony morphology included colony characteristics on NA media and cell morphology (cell shape, Gram reaction, and motility). The biochemical characteristics examined included gelatin hydrolysis, catalase activity, and carbohydrate fermentation of various sugars (sucrose, glucose, maltose, and lactose). The physiological characteristics analyzed included oxygen requirements, temperature tolerance, NaCl tolerance, and pH tolerance [1][9].

3. Results and Discussion

3.1. Characteristics of Kendari Bay Sediments

Kendari Bay Sediments are blackish mud with organic carbon (C-organic), total nitrogen (N-total), power of hydrogen (pH), and electrical conductivity (EC) characteristics listed in Table 1.

Table 1. Characteristics of Kendari Bay Sediment substrate before and after SMFC application

Test Parameters	Substrate Before SMFC	Substrate After SMFC
C-organic	4.23%	4.06%
N-total	1.08%	0.97%
C/N	3.92	4.19
EC	11.56 mS	15.78 mS
pH	7.38	7.86

Table 1 shows that the organic carbon content of Kendari Bay sediments is relatively high and comparable to that of closed ecosystems such as lakes. This condition is related to the accumulation of organic matter influenced by organic material input, sedimentation rates, and slow degradation processes. Physically, Kendari Bay has characteristics similar to those of an estuary, where freshwater supply from upstream rivers and tidal influences contribute to high C-organic content. Total nitrogen content is also an essential parameter for determining substrate characteristics in the sediment microbial fuel cell (SMFC) system. At the same time, the C/N ratio is used as a simple indicator to assess the decomposition rate [8][10]. The C/N ratio of Kendari Bay marine sediments was recorded at 3.92, indicating the occurrence of nitrogen mineralization, namely the conversion of organic nitrogen into inorganic forms by microbial activity. A C/N ratio below 15 generally indicates a high decomposition rate, thereby supporting microbial activity in SMFC [11]. In addition, the sediment pH value, which ranges from 6 to 8, is within the optimal range for nutrient availability and decomposer bacterial activity. Sediment conductivity of 11.56 mS reflects seawater's high mineral and ion content, which contributes to increased electrical conductivity of the electrodes in the SMFC system [12].

After being used in the SMFC system, the sediment showed a visual change from black to brown, where black generally represents a higher organic content. The data in table 1 indicate a decrease in organic content after the sediment was used as an SMFC substrate, as shown by the reduction in carbon and nitrogen levels in line with the pattern of electricity production. This decrease is related to microbial activity degrading the sediment's organic matter. Organic carbon content decreased from 4.23% to 4.06%, while total nitrogen levels decreased from 1.08% to 0.97% [13]. In addition, after utilization in SMFC, the pH and electrical conductivity of the sediment increased. The increase in pH indicates a faster transfer of protons to the cathode. In contrast, the increase in electrical conductivity is caused by the polarization of organic compounds in the sediment into simple ions such as butyric acid, propionic acid, and acetic acid [14][15].

3.2. Electrical Energy Generated by Kendari Bay Marine Sediments

Measurement of the SMFC system began by recording the open-circuit voltage at 2-hour intervals for 24 hours, until the system reached a stable condition [1][16]. The results are shown in Figure 2.

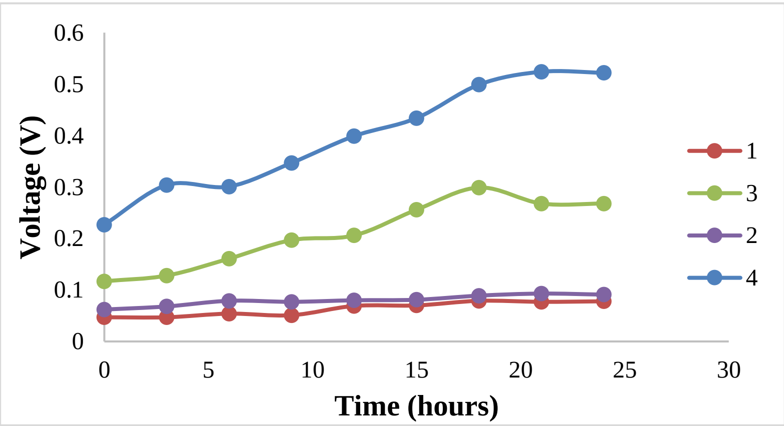


Figure 2. Comparison of time and voltage (open circuits voltage) generated by Kendari Bay Sediment SMFC

Description:

1. Single SMFC open circuit voltage of Kendari Bay Sediments using aerators
2. Single SMFC open circuit voltage of Kendari Bay Sediment using $KMnO_4$
3. Multiple SMFC open circuit voltage of Kendari Bay Sediments using aerators
4. Multiple SMFC open circuit voltage of Kendari Bay Sediment using $KMnO_4$

Based on Figure 2, the open-circuit voltage in the four SMFC systems initially increased and then stabilized at the measurement time, approximately 18 hours. This indicates that the microbes were in a phase of consistent metabolic activity. The open-circuit voltage showed differences in measurement results between single cells and double cells, using aerators and potassium permanganate ($KMnO_4$). In single MFC cells, the voltage generated reached 0.078 V (aerator) and 0.091 V ($KMnO_4$), while in double MFC cells, it increased to 0.268 V (aerator) and 0.522 V ($KMnO_4$). The use of $KMnO_4$ as an electron acceptor at the cathode was proven to improve SMFC performance compared to oxygen aeration. This can be explained in terms of redox potential and its compatibility with electrogenic microbial activity. Electrochemically, the MnO_4^-/Mn^{2+} redox pair has a higher standard potential than the O_2/H_2O pair under acidic conditions. This potential difference provides a greater thermodynamic driving force in the process of electron transfer from the anode to the cathode, resulting in a higher voltage [17].

After measuring the open-circuit voltage, the SMFC voltage was measured daily for 18 days for the aerator system and 31 days for $KMnO_4$, corresponding to the duration of microbial activity producing electrical energy [18], as shown in Figure 3.

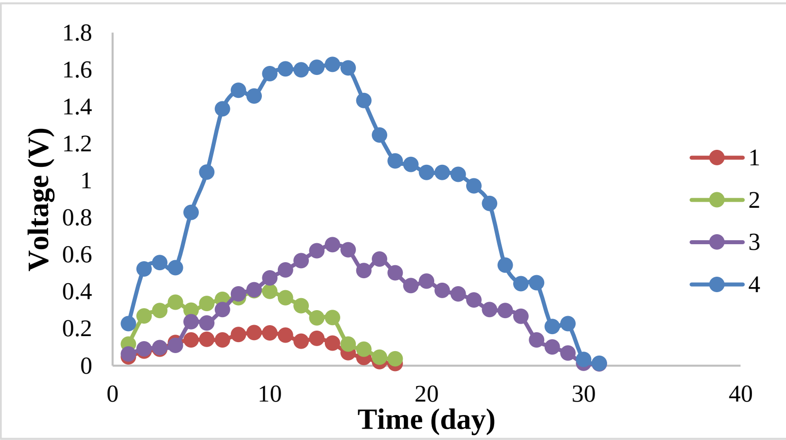


Figure 3. Comparison of time and voltage generated by Kendari Bay Sediment SMFC

Description:

1. Single MFC voltage of Kendari Bay marine sediments using an aerators
2. Multiple MFC voltage of Kendari Bay marine sediments using an aerators
3. Single MFC voltage of Kendari Bay marine sediments using $KMnO_4$
4. Multiple MFC voltage of Kendari Bay marine sediments using $KMnO_4$

Based on Figure 3, the highest SMFC peak voltage was achieved by five cells with $KMnO_4$ at 1.628 V (day 14), while four cells with aerators reached 0.404 V (day 9). In one cell, the maximum voltage was 0.653 V ($KMnO_4$) and 0.178 V (aerator). The higher voltage in the $KMnO_4$ system was due to a greater oxidation potential compared to O_2 and a parallel cell configuration that increased the stability of electron distribution [19][20]. In addition, the sediments of Kendari Bay have a fairly high conductivity (11.56 mS) and a low C/N ratio (3.92), which supports electrogenic microbial activity and electron transfer efficiency. The decrease in voltage at the end of the measurement period was due to a reduction in the organic substrate at the anode [21].

Based on the results of Analysis of Variance (ANOVA) using Jamovi software with a confidence level of 95%, differences in the type of electron acceptor (aerator and $KMnO_4$) and the number of parallel cells were found to have a significant effect on the electrical voltage produced by the SMFC system ($p < 0.05$). The highest voltage was achieved in the SMFC system with five parallel cells and $KMnO_4$, which was significantly different from the other treatments. This demonstrates that increasing the number of parallel cells and using $KMnO_4$ enhances the electrochemical driving force, resulting in a higher output voltage.

The measurement results show that the addition of $KMnO_4$ produces a higher SMFC current compared to the aerator, with current detected only in the double cell circuit. This finding is consistent with the recommendations of Oh & Logan [22], as well as Arsov & Georgievski [23], regarding the combination of cells to increase current output. The initial current reached 5.0 µA and decreased to 2.1 µA within 5 days. The relationship between voltage and SMFC current is shown in Figure 4.

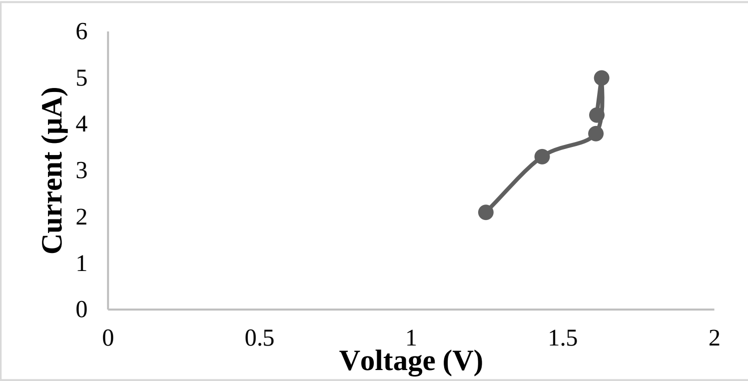
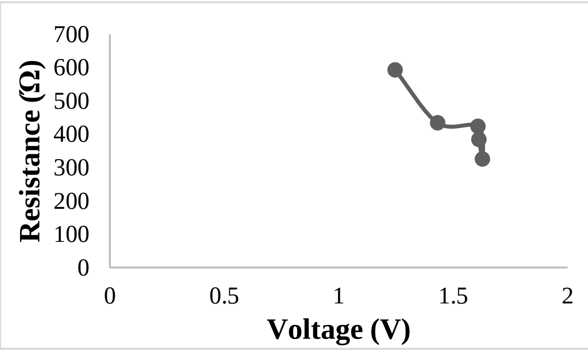
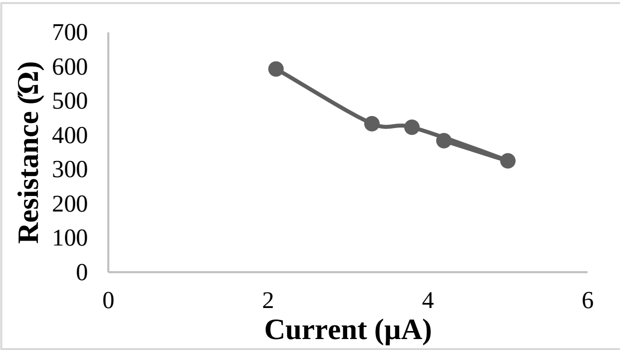


Figure 4. Comparison of voltage and current generated by Kendari Bay Sediment SMFC

The voltage and current in the SMFC system exhibit a direct proportional relationship, in line with the findings of Ewing et al. [24] and Ye et al. [25], which demonstrate that the addition of electrolyte to the cathode increases both current and voltage. The low current is caused by high internal resistance in the circuit, as shown in Figure 5.





Figures 5. **(a)** Comparison of voltage and current generated by Kendari Bay Sediment SMFC
(b) Comparison of current and resistance generated by Kendari Bay Sediment SMFC

In addition to generating voltage and current, the performance of SMFC systems is generally also assessed based on internal resistance and coulombic efficiency parameters [26]. In this study, the relatively small current value (5.0 µA when using $KMnO_4$) with a maximum voltage of 1.628 V indicates that the system has a fairly high internal resistance. This resistance may originate from the distance between the electrodes and the resistance in the salt bridge. This is consistent with the results shown in Figure 5, where an increase in resistance is accompanied by a decrease in current and voltage, indicating that electron transfer efficiency remains suboptimal. The lowest resistance value obtained was 325.6 Ω, resulting in a low current output.

Coulombic efficiency (CE) remains low. This is indicated by a decrease in organic carbon (from 4.23% to 4.06%) and total nitrogen (from 1.08% to 0.97%) that is not entirely converted into electrical current. Some of the electrons resulting from organic degradation are likely used for non-electrogenic microbial respiration or the reduction of other compounds present in the sediment, such as sulfate or nitrate. Thus, even though the system successfully generates electrical energy, the proportion of chemical energy that is actually converted into electrical current is still limited [27].

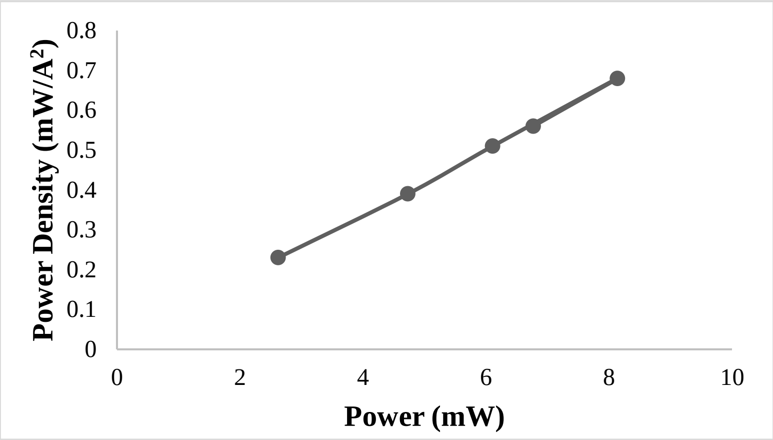


Figure 6. Comparison of power and power density generated by Kendari Bay Sediment SMFC

The power density produced by the SMFC system is relatively low because the electrical current obtained is also low and is not directly proportional to the voltage produced. This suggests that the system's efficiency in converting electrical energy remains limited. Power density is greatly influenced by the combination of current and voltage, so that a decrease in either parameter will have a direct impact on the total power generated. Factors such as internal cell resistance, electron transfer efficiency, and microorganism activity on the electrodes can also contribute to the low overall performance of the SMFC system [28].

3.3. Bacterial Characteristics of Kendari Bay Sediments

The study successfully grew four bacterial colonies, which were then identified numerically based on their morphology, physiology, and biochemistry. Because morphological similarities can occur between species, physiological and biochemical characteristics are important determinants in identification [9]. Identification was carried out through biochemical testing and Gram staining, with the results of the characterization of SMFC bacterial isolates presented in Table 2.

Table 2. Biochemical characteristics of SMFC bacterial isolates

Isolate Type	Fermentation				Catalase	Gelatinase	Motility
	Glucose	Maltose	Lactose	Sucrose			
S_1	positive	positive	negative	positive	positive	positive	positive
S_2	positive	positive	negative	positive	positive	positive	positive
S_3	positive	positive	positive	negative	positive	positive	negative
S_4	positive	negative	negative	negative	positive	positive	positive

Table 2 data show that SMFC bacterial isolates ferment carbohydrates to produce organic acids (acetate, lactate, formate, and succinate) and gases (CO_2 and H_2). Electrons from this process are transferred to the SMFC anode, generating electrical energy through the fermentation of glucose [29].

$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$

Carbohydrate fermentation produces organic acids that lower the pH, indicated by a color change in the bromothymol blue (BTB) indicator from red to yellow. The final fermentation products depend on the characteristics of each bacterial isolate.

The catalase test is performed to detect the presence of the catalase enzyme in bacterial isolates. This enzyme is commonly found in many types of bacteria and plays a role in the oxidation process by breaking down hydrogen peroxide into water and oxygen [30].

$2H_2O_2 \xrightarrow{\text{catalase enzyme}} 2H_2O + O_2$

The catalase test is crucial for determining the nature of bacteria in terms of their oxygen requirements. The catalase test revealed that all SMFC bacterial isolates were catalase-positive, as evidenced by the production of bubbles upon the addition of H_2O_2. This indicates that the bacteria are aerobic.
The gelatinase test examines the ability of bacteria to break down gelatin with the exoenzyme gelatinase. The melting of the medium indicates hydrolysis after inoculation of the bacterial isolate [31]. The results of the study show that all isolates were able to hydrolyze gelatin positively. The results of the bacterial motility test indicate that all SMFC bacterial isolates are motile, meaning they possess flagella as their means of movement.

Furthermore, the four isolates were identified through Gram staining and observation of colony morphology to distinguish between Gram-positive and Gram-negative bacteria. The morphological details of each isolate are presented in Table 3.

Table 3. Characteristics of SMFC bacteria based on shape and Gram staining of Kendari Bay Sediments

Isolate Type	Colony Shape	Colony Color	Cell Shape	Gram Staining
S_1	circular	yellow	basil	positive
S_2	circular	white	basil	positive
S_3	circular, serrated	white	basil	positive
S_4	circular, serrated	yellow	basil	positive

Table 3 shows SMFC bacterial isolates from Kendari Bay that are Gram-positive, rod-shaped, and suspected to belong to the genus Bacillus. This genus plays a crucial role in decomposing organic matter into simple nutrients, thereby supporting nutrient recycling in sediments [32]. Bacillus subtilis and Bacillus pumilus are common species that can form spores to survive in extreme conditions.

4. Conclusion

The sediments of Kendari Bay demonstrate strong potential for generating electrical bioenergy through SMFC technology, with a measured voltage of 1.628 V exceeding the average voltage of a standard 1.5 V battery. This voltage was obtained from an SMFC system arranged in series and measured in a parallel configuration. The performance is supported by the organic content of the sediments, consisting of 4.23% organic carbon and 1.08% total nitrogen, which provide sufficient substrates for microbial activity and electron transfer. These results indicate the feasibility of utilizing Kendari Bay sediments as a sustainable bioenergy source.

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