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THE ROLE OF EM4 (EFFECTIVE MICROORGANISMS) IN SOLID WASTE-POWERED MICROBIAL FUEL CELLS: INVESTIGATING VOLTAGE OUTPUT AND ELECTRICAL CONDUCTIVITY FOR BIOELECTRICITY GENERATION

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Abstract:
The increasing global demand for renewable energy and sustainable waste management solutions has inspired interest in microbial fuel cells (MFCs) as a dual-purpose technology for bioelectricity generation and waste treatment. This study explores the role of EM4, a consortium of effective microorganisms, in enhancing the voltage output and electrical conductivity of solid waste-powered MFCs. A batch system bioreactor assessed the impact of varying organic waste-to-zeolite ratios on MFC performance. The results demonstrated that a 1:1 ratio of organic waste to zeolite produced the highest electrical conductivity (3160 µS/cm) and the most substantial voltage output (777.5 mV) by day three of the experiment. Statistical analysis, including ANOVA and Kruskal-Wallis tests, revealed significant differences in voltage output across treatments, with a positive correlation between electrical conductivity and voltage production. These findings highlight the potential of integrating EM4 and conductive materials like zeolite to optimize bioelectricity generation in MFCs, contributing to the advancement of sustainable energy technologies.
Keywords: Electrical Conductivity, EM4, MFCs, Solid Waste, Voltage

INTRODUCTION

The global demand for renewable energy sources has intensified in recent years due to the growing concerns over environmental degradation and the depletion of fossil fuels. Among the various alternative energy technologies, microbial fuel cells (MFCs) have gained significant attention for their dual role in generating bioelectricity while simultaneously treating organic waste. MFCs utilize the metabolic activities of microorganisms to convert chemical energy from organic substrates directly into electrical energy, offering a sustainable solution for energy recovery from waste materials (Zarena, 2023). In recent years, MFC technology has garnered attention for its potential to address two critical environmental issues: the generation of renewable energy and the treatment of wastewater and solid waste (Gurikar et al., 2021; Elhenavy et al., 2022; Parwate et al., 2024).

In recent years, the application of Effective Microorganisms (EM4) in MFCs has emerged as a promising technique to enhance the efficiency of bioelectricity production. EM4, a consortium of beneficial microorganisms, has been widely recognized for accelerating organic matter decomposition and enhancing microbial activity in various biotechnological applications (Safwat & Matta, 2021). Its potential to improve the performance of MFCs, particularly in terms of voltage output and electrical conductivity, makes it a valuable tool in renewable energy research.

Solid waste, particularly from urban sources, represents a largely untapped source of organic material for MFC systems. By integrating EM4 into MFCs powered by solid waste, the biological activity of the microorganisms can be optimized, potentially leading to increased bioelectricity generation. However, the efficiency of MFCs in converting waste into electrical energy depends on various factors, including substrate composition, microbial activity, and the electrochemical properties of the system. Among these, voltage output and electrical conductivity play critical roles in determining the overall performance of MFCs.

Voltage output reflects the potential difference generated by the microbial metabolic processes, and optimizing this output is essential for improving the overall energy yield of the system. On the other hand, electrical conductivity influences the efficiency of electron transfer within the MFC, affecting the resistance and overall energy conversion efficiency. Research on carbon felt (CF) anodes, commonly used for their electrical conductivity and surface area, found that surface modifications can enhance electron transfer efficiency. Improved conductivity and charge transfer were achieved by coating the anode with carbon nanofibers (CNFs), leading to better biofilm formation and electron transfer (Ghanam et al., 2023). Another research on plant-microbial fuel cells (P-MFCs) has shown that the internal resistance of MFCs can vary based on environmental factors, such as the presence of plant roots. The study highlights how internal resistance reduction through optimized materials or system configurations can improve electron flow, enhancing voltage output and electrical conductivity (Martinez, 2024). By understanding and optimizing Voltage and electrical conductivity parameters, it is possible to enhance the bioelectricity generation capacity of MFCs when solid waste is used as a substrate.

This study aims to investigate the role of EM4 in enhancing the performance of solid waste-powered MFCs. Specifically, it evaluates MFC systems' voltage output and electrical conductivity utilizing EM4. These parameters are critical in assessing the efficiency of MFCs as bioelectricity generators. The findings will improve the design and operational conditions of MFCs, enhancing their viability as a sustainable energy solution in the context of solid waste management.

METHODS

Bioreactor Preparation. The bioreactor used in this study was designed as a batch system to facilitate the conversion of chemical energy from solid waste into electrical energy under anaerobic conditions. The MFC bioreactor leverages microbial metabolism to produce electricity. This batch system allows the complete microbial growth cycle, ensuring the microbes transition through the lag, log, and death phases. The bioreactor was constructed from glass materials to avoid unwanted chemical reactions with the waste material. The bioreactor design ensures that the microbial communities operate optimally throughout the experiment, producing efficient bioelectric production. The schematic representation of the bioreactor is provided in Figure 1.

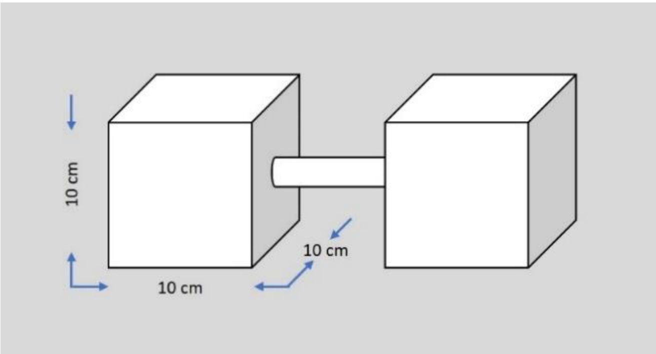

Figure 1. MFC System

The MFC bioreactor was constructed with two compartments, each made of glass with a volume of 1000 cm^3. These compartments serve as the anode and cathode chambers. The anode

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compartment operates under anaerobic conditions, while the cathode compartment is aerobic. A proton exchange membrane (PEM) separates the two chambers, allowing protons generated in the anode compartment to diffuse into the cathode compartment. The protons combine with oxygen to produce water, completing the electrochemical process. Solid waste was used as the substrate in the anode compartment of the MFC, while the cathode compartment was filled with a 0,5% NaCl solution. Adding NaCl in the cathode increases the electrolyte's conductivity, facilitating the efficient flow of ions, mainly protons, through the proton exchange membrane. This enhanced ion transfer reduces the internal resistance of the system, leading to improved overall performance of the microbial fuel cell.

Electrode Preparation. We used graphite rods sourced from unused batteries as electrodes in the anode and cathode compartments of the microbial fuel cell (MFC) system. Employing graphite rods derived from battery waste in MFCs is a sustainable approach that promotes materials recycling while reducing waste. This method offers a cost-effective alternative to commercially available electrodes and aligns with environmental conservation efforts by repurposing discarded battery components. By integrating waste graphite into MFCs, we aim to balance performance with sustainability, contributing to developing eco-friendly bioelectrochemical systems.

Membrane Preparation. In the laboratory, an artificial membrane was created using a combination of chitosan and agar. Chitosan was extracted from the shells of vannamei shrimp (Litopenaeus vannamei) following deproteination, demineralization, and deacetylation. The deproteination process began with grinding the shrimp shells soaked in a 3.5% NaOH solution at 65°C for 120 minutes. Afterward, the shells were thoroughly rinsed with water until neutral pH was achieved, then dried in an oven at 65°C for 24 hours. In the demineralization step, the deproteinated shells were immersed in a 4% HCl solution, stirred, and heated at 30°C for 60 minutes. The shells were rinsed with distilled water until neutral pH and dried at 65°C for 24 hours, resulting in chitin extraction. The final step, deacetylation, involved treating the chitin with 50% NaOH at 100°C for 4 hours, with constant stirring. The resulting solid, chitosan, was washed with distilled water until neutral pH, dried in an oven, and ground into a fine powder (Hosney et al., 2022). Finally, 10 grams of this chitosan powder were mixed with 1 gram of agar to complete the preparation.

Solid Waste Preparation. We utilized a 1% concentration of EM4 (Effective Microorganisms) as a microbial inoculum in the anode compartment to enhance the degradation of organic materials. EM4 is a widely recognized microbial consortium containing diverse beneficial microorganisms, including lactic acid, yeast, actinomycetes, and photosynthetic bacteria. These microbes work synergistically to accelerate the breakdown of complex organic matter into simpler compounds. By facilitating efficient microbial activity, EM4 improves the rate of organic degradation, subsequently releasing electrons into the MFC system. This electron flow contributes to the bioelectricity generation process, enhancing the overall efficiency of the microbial fuel cell.

Microbe Preparation. We utilized a 1% concentration of EM4 (Effective Microorganisms) as a microbial inoculum in the anode compartment to enhance the degradation of organic materials. EM4 is a widely recognized microbial consortium containing diverse beneficial microorganisms, including lactic acid, yeast, actinomycetes, and photosynthetic bacteria. These microbes work synergistically to accelerate the breakdown of complex organic matter into simpler compounds. By facilitating efficient microbial activity, EM4 improves the rate of organic degradation, subsequently releasing electrons into the MFC system. This electron flow contributes to the bioelectricity generation process, enhancing the overall efficiency of the microbial fuel cell.

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Voltage Measurement. Voltage output, the potential difference produced in the MFC system, was measured at three intervals daily (morning, afternoon, and evening) over three consecutive days. A voltmeter was used to accurately capture the fluctuations in Voltage for each waste composition treatment. This methodology allowed the researchers to track the performance of the MFC over time, highlighting trends in bioelectricity production based on the organic and inorganic waste ratios.

Electrical Conductivity Measurement. In addition to voltage measurements, the study incorporated the electrical conductivity analysis to assess the efficiency of the MFC system in generating bioelectricity. Electrical conductivity was measured to determine the ion concentration in the system, which can influence microbial performance and electron transfer efficiency. These measurements were taken on the third day of the experiment to capture the system's performance at a stable stage. For this analysis, the research team collaborated with PDAM Surya Sembada Surabaya, the local water utility agency in Surabaya, Indonesia, to ensure precise and accurate measurements.

Data Collection. The analysis results were examined using descriptive analysis. This approach aims to describe the findings related to bioelectricity production and electrical conductivity. In this process, the results are compared with those from previous studies conducted by other researchers to provide context and insights.

Statistical Analysis. Statistical analysis was conducted using R to differentiate between treatments, assess time-related effects, and examine the correlation between electrical conductivity and Voltage.

RESULT AND DISCUSSION

This study investigated the effect of varying solid waste compositions and the addition of conductive materials on microbial fuel cell (MFCs) performance in bioelectricity generation.

Electrical Conductivity. Figure 2 presents the average electrical conductivity values for each treatment.

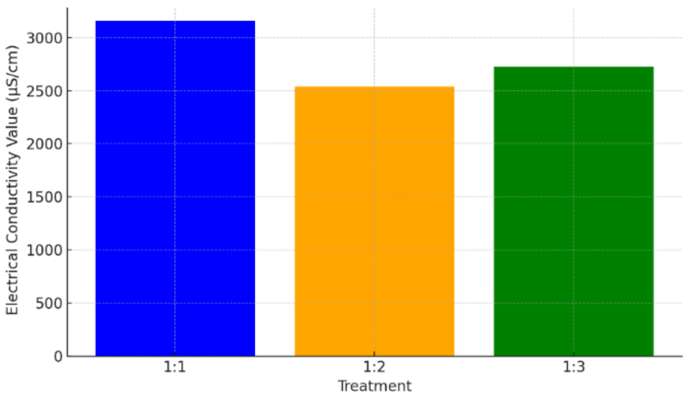

Figure 2. Electrical Conductivity by Treatment

The results indicate that the average electrical conductivity for treatment 1:1 is the highest at 3160 μS/cm, suggesting it is the most effective in enhancing ion transfer and reducing resistance within the system. In contrast, treatment 1:2 has the lowest average conductivity value at 2540 μS/cm, indicating lower efficiency in electrical conduction. Treatment 1:3 shows moderate performance, with an average conductivity value of 2725 μS/cm. Overall, the data suggest that the

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composition used in treatment 1:1 creates a more favorable electrical conductivity environment than the other treatments. Natural zeolite has been utilized in MFC setups for its ion exchange properties, which creates a favorable environment for ion transport and enhances electron transfer. One study showed that incorporating zeolite into an MFC significantly increased power density (Venkatesan & Dharmalingam, 2015).

Voltage Output. The following graph represents the voltage values measured over time for different treatment groups in a microbial fuel cell (MFC) experiment. Voltage measurements were taken on the first and third days to observe the changes in electrical output at various times throughout the day (morning, afternoon, and evening). The three treatment groups (1:1, 1:2, and 1:3) represent different ratios of organic waste and zeolite used in the MFC system.

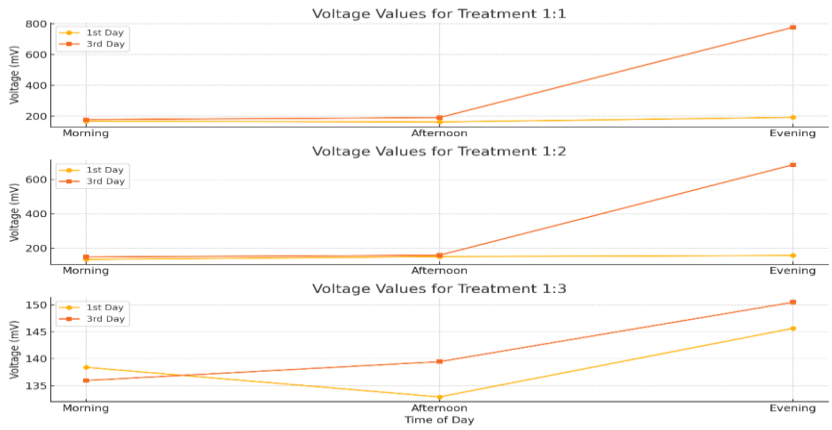

Figure 3. Voltage Values Over Time for Different Treatments (1st day and third day)

The graph shows that treatments 1:1 and 1:2 exhibit substantial increases in voltage from the 1st day to the 3rd day, particularly in the evening, where the voltage rises sharply to 777.5 mV and 685.5 mV, respectively. In contrast, treatment 1:3 shows much less variation in voltage between the two days, with relatively stable values around 135-150 mV across all time points. On the 1st day, all treatments start at lower voltage levels, ranging from 130 mV to 190 mV, with minimal variation throughout the day. However, by the 3rd day, treatments 1:1 and 1:2 see large voltage spikes by the evening, while treatment 1:3 remains almost unchanged. This indicates that treatments 1:1 and 1:2 potentially create more favorable conditions for microbial activity or electron transfer as the system develops, leading to increased bioelectricity production. Treatment 1:3, however, might not provide the same conditions for enhanced microbial performance, reflected in its steady voltage output (Prasetyadewi et al., 2024). Studies have shown that incorporating zeolite enhances ion mobility, reduces internal resistance, and improves power output over time. These materials create a more conducive environment for microbial interactions, increasing bioelectricity production as the system matures (Venkatesan & Dharmalingam, 2015; Paul et al., 2018). On the other hand, higher ratios of zeolite, such as 1:3, may not provide the same enhancements in conductivity or microbial performance as reflected by a more stable but lower voltage output. This may be due to the excessive adsorption properties of zeolite, which can limit nutrient availability or ion exchange, slowing microbial activity and electron transfer (Zarena, 2023).

Statistical Analysis. Since the data for electrical conductivity follows a normal distribution, as shown in Table 1, we analyzed the variable differences using ANOVA. Table 2 represents the results

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of a one-way ANOVA test to compare the effects of different treatment ratios on electrical conductivity in the microbial fuel cell (MFC) system.

Table 1. Shapiro-Wilk Normality Test for Electrical Conductivity

Test	W-Statistic	p-value	Interpretation
Shapiro-Wilk Normality Test	0.93541	0.6225	Data is normally distributed ($p > 0.05$)

Source: Data Processed 2024

Table 2. ANOVA Result on Electrical Conductivity

		Df	Sum Sq	Mean Sq	F value	Pr (>F)
1	Treatment	2	405233	202617	1.182	0.418
2	Residuals	3	514450	171483		

Source: Data Processed 2024

The ANOVA results indicate that there is no significant difference in electrical conductivity between the three treatments (1:1, 1:2, 1:3) with a p-value of 0.418 (>0.05). Moreover, the data indicate that the average electrical conductivity for treatment 1:1 is the highest at 3160 µS/cm compared to other treatments. Meanwhile, since the voltage output data in the MFC did not follow a normal distribution for the entire dataset (Table 3), we performed a Kruskal-Wallis's test to compare the treatments. Table 4 represents the results of Kruskal-Wallis's test, while Figure 3 represents the results of pairwise comparisons from Dunn's test.

Table 3. Shapiro-Wilk Normality Test for Voltage Value

Treatment	Shapiro-Wilk p-value	Conclusion
1:1	2,26E-05	Not Normally Distributed
1:2	2,07E-05	Not Normally Distributed
1:3	0,536265	Normally Distributed

Source: Data Processed 2024

Table 4. Kruskal-Wallis Results on Voltage Value

Comparison	Chi-squared	df	p-value	Conclusion
Voltage by Treatment	21,103	2	2,62E-05	A significant difference between treatments
Voltage by Day	2,7078	1	0,09986	No significant difference between 1st and third day
Voltage by Time	21,103	2	2,62E-05	A significant difference between periods

Source: Data Processed 2024

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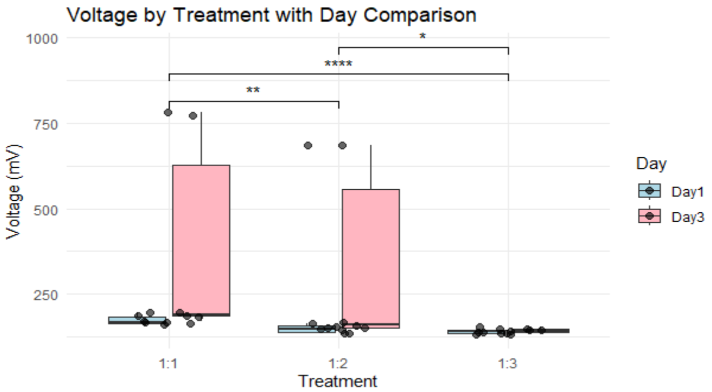

Figure 4. Results of Pairwise Comparisons from Dunn's Test

Significant differences exist between the treatments, particularly on Day 3, where treatments 1:1 and 1:2 show higher voltage outputs than treatment 1:3. The most substantial changes in Voltage occur in treatments 1:1 and 1:2, with much higher voltage values on Day 3. In contrast, treatment 1:3 does not exhibit the same significant voltage increase, indicating it may be less effective than the other treatments. A similar study demonstrated that smaller feed particle sizes in microbial fuel cells (MFCs) resulted in higher power densities than those with larger particle sizes (Parwate et al., 2024). This suggests that the 1:3 treatment might contain larger particle sizes from organic waste and zeolite, which could hinder efficient electron transfer and reduce power density. The larger particles may limit surface area exposure, slowing microbial activity and decreasing overall system performance.

Moreover, using EM4 bacteria, primarily designed to degrade organic material, plays a significant role in this dynamic. In treatments with higher zeolite content, such as the 1:3 ratio, the metabolic activity of EM4 bacteria could be compromised. Since zeolite is not an organic substrate, the higher proportion of zeolite in the system likely reduces the amount of organic material available for the bacteria to metabolize. As a result, the bacteria's efficiency in generating electrons through the degradation process decreases, leading to lower voltage outputs. The EM4 consortium is a blend of beneficial microorganisms working together to degrade organic material efficiently. Lactic acid bacteria (LAB) initiate the process by breaking down sugars and producing lactic acid, creating a favorable acidic environment. Photosynthetic bacteria contribute by converting carbon dioxide into organic compounds, indirectly supporting microbial activity. Yeast ferments sugars into alcohol and carbon dioxide, facilitating further decomposition. Actinomycetes and fermenting fungi are crucial for degrading complex plant-based materials like cellulose and lignin. Nitrogen-fixing bacteria enrich the microbial ecosystem by converting atmospheric nitrogen into usable forms, enhancing the overall decomposition process (Safwat & Matta, 2021). Together, these microbes form a synergistic system that optimizes the breakdown of organic waste in applications like microbial fuel cells (MFCs). This study demonstrates that EM4 is highly effective in metabolizing organic material to produce bioelectricity through a Microbial Fuel Cell (MFC) system. While electrogenic bacteria are typically known for generating electricity in MFCs, the study highlights that any bacteria capable of metabolizing compounds to release electrons can be harnessed in this system (Herawati et al., 2022). EM4, which contains a diverse microbial consortium, proves particularly capable of producing higher voltage outputs compared to specific electrogenic bacteria like *Achromobacter xylosoxidans*, which generated a peak voltage of 1.01 ± 0.06 V on day 24 (De La Cruz-Noriega et al., 2023). The enhanced voltage production with EM4 suggests that the synergistic activity of its diverse

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bacteria, including lactic acid, photosynthetic, and yeast, may contribute to more efficient electron release and bioelectricity generation than single-species electrogenic systems (Eksandy, 2024).

We further analyzed the relationship between electrical conductivity and Voltage in the microbial fuel cell system. Figure 4 shows a clear positive correlation between Voltage (mV) and Electrical Conductivity (µS/cm).

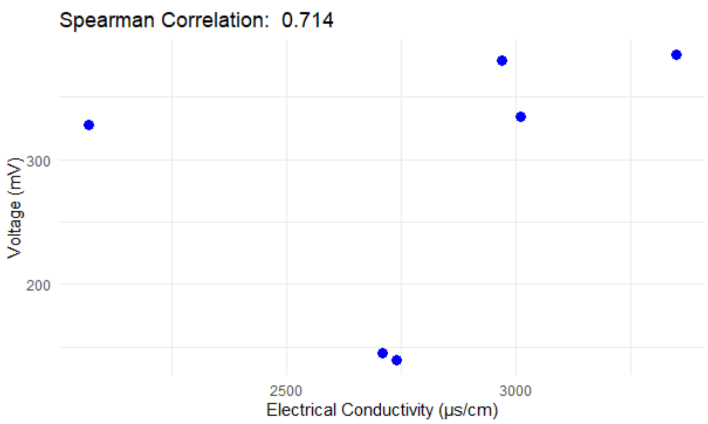

Figure 5. Results of the Spearman Correlation Test

The Spearman correlation coefficient of 0.714 suggests a solid positive monotonic relationship, indicating that as electrical conductivity increases, Voltage also rises. This supports the hypothesis that improving the system's electrical conductivity could lead to enhanced voltage production in the microbial fuel cell. Optimizing materials and configurations to enhance conductivity—such as using advanced catalysts and biofilms—has boosted MFC performance (Khater et al., 2021; Bashir et al., 2021; Calderon et al., 2020).

CONCLUSION

This study demonstrates the effectiveness of integrating EM4 (Effective Microorganisms) with solid waste-powered microbial fuel cells (MFCs) for enhanced bioelectricity generation. The results show that a 1:1 ratio of organic waste to zeolite provides the most favorable conditions for electrical conductivity and voltage output, suggesting that the combination of EM4 and conductive materials such as zeolite can significantly improve the performance of MFCs. The higher voltage output and electrical conductivity observed in this configuration underline the importance of optimizing substrate composition to promote microbial activity and efficient electron transfer. Overall, applying EM4 in MFC systems offers a promising approach to enhancing bioelectricity production from organic waste, making it a viable and sustainable solution for energy recovery in waste management. Future research should explore long-term stability, scalability, and material optimizations to improve the commercial feasibility of MFCs in real-world applications.

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