Exploring the Potential of Stevia Fermentation Liquid and Wastewater in Microbial Fuel Cells: A Sustainable Approach to Enhanced Electricity Production

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Abstract – In response to the growing need for eco-friendly and sustainable energy sources, it is crucial to explore alternative solutions. Microbial fuel cells, or MFCs, provide a promising avenue for electricity production. In this paper, the study aims to find the most efficient combination of microorganisms and organic matter for enhanced electricity production under environmental conditions. A series of experiments found that the combination of stevia and wastewater at 25˚C and no exposure to UV light proved to be most efficient in electricity production, even when compared to the control variable of Shewanella. The findings underscore the feasibility of utilizing Stevia fermentation liquid and wastewater in MFCs as a sustainable alternative to conventional energy sources, providing insights for future developments in environmentally friendly solutions.

Keywords: alternative energy source, electricity production, microbial fuel cell, stevia, sustainable energy
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1.Introduction

Despite efforts to diversify energy sources, it remains a stark reality that a significant portion of the world relies heavily on energy generated through the combustion of fossil fuels. This practice presents as unsustainable, and mass produces carbon dioxide, intensifying the greenhouse effect and accelerating global warming.

In alignment with the growing awareness of the detrimental effects of carbon emissions, exploring alternative sources to mitigate this impact while meeting the heightened global demand for electricity is imperative. One such avenue is the development of Microbial Fuel Cells (MFCs), which are bioelectrochemical devices that utilize the metabolic activities of microorganisms to convert the chemical energy in organic matter into the release of electrons and, thus, electrical current [1].

1.1.Background Information: Microbial Fuel Cells

A typical MFC consists of three main components: a cathode chamber, an anode chamber, and a separator. These chambers are connected by a proton exchange membrane (PEM) to allow the protons produced from the anode to pass through to the cathode chamber, which is equipped with an electrical circuit, such as copper wires, to facilitate the flow of electrons. The PEM selectively permits the passage of protons while preventing the mixing of gases and other substances between the chambers. The microorganisms in the anode chamber oxidize the organic compound to produce electrons, protons, and carbon dioxide [2].

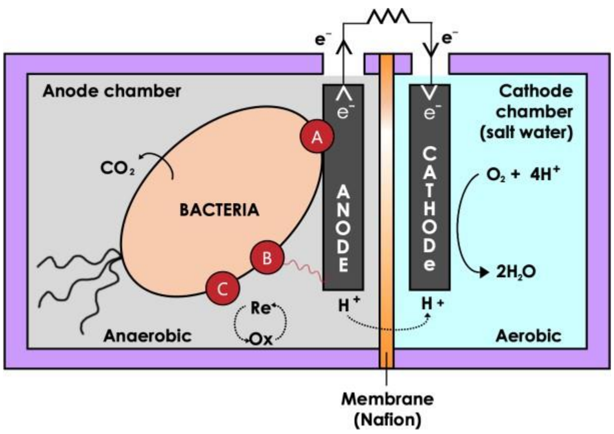

Figure 1. Diagram representing a microbial fuel cell (MFC), illustrating key components and electron flow [3]

Electrons are then transferred by microbes to the anode chamber and flow to the cathode chamber through a resistor, producing electricity. The protons also travel through the electrolyte to the cathode chamber, where they partake in an oxygen-reducing reaction to OH^- or H_2O [2]. At the cathode, electrons combine with protons and an external electron acceptor, often oxygen, to complete the electrochemical circuit, forming water as a byproduct. The following equations, using acetate, demonstrate the reactions in the anode and cathode chambers:

$$Anode\ Chamber: CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$$

$$Cathode\ Chamber: 2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O$$

1.2. Background Information: Stevia

Stevia, or Stevia rebaudiana Bertoni, is a perennial herb native to the Asteraceae family. Its native habitat is the South American border region between Paraguay and Brazil. In this region, Stevia is called "Dulce" and has been used for centuries by grinding its leaves into powder to sweeten beverages such as mate tea or coffee.

Antioxidant action refers to the ability to reduce or inhibit the generation of reactive oxygen species produced in the metabolic processes of cells in plants and animals. Reactive oxygen species are associated with aging, atherosclerosis, cancer, and other health issues. These highly oxidizing oxygen remnants, generated when the oxygen we breathe is converted to energy and reduced to water, can also result from factors such as stress, ultraviolet radiation, and bacterial invasion.

Moderate levels of reactive oxygen species are essential for protecting the body from bacteria and foreign substances. However, excessive production can accumulate peroxidized lipids, significant components of cell membranes, attacking the body and becoming a culprit in various adult diseases and aging.

Stevia extracts contain various compounds that exhibit antioxidant action. Particularly noteworthy is the presence of diverse polyphenols, with Stevia found to have the highest amount of polyphenols, including a total of 75 types, among various herbs. Moreover, Stevia is known to possess over 20 times higher antioxidant activity than green tea and demonstrates superior antioxidant ability compared to the potent antioxidant Trolox in experimental comparisons. Due to its antioxidant capabilities, selective antimicrobial effects, and the ability to break down environmental hormones and harmful substances, Stevia is being utilized as an eco-friendly agricultural material.

Stevia fermented extract is a concentrated, fermented liquid obtained from Stevia leaves and stems extraction. It enhances the sweetness and unique flavor of various vegetables and fruits while extending their shelf life.

Additionally, it exhibits excellent decomposition effects on environmental hormones, pesticides in the soil, and dioxins, making it suitable for use as a soil microbial agent in environmentally friendly farming practices. Recent studies have identified and characterized effective microorganisms separated from the fermented extract, shedding light on their taxonomic classification. However, despite numerous effective experimental findings related to Stevia extract, the specific components responsible for these effects have not yet been fully explored.

1.3 Objective of research

Further research must be conducted to potentially implement microbial fuel cells as sustainable alternatives to the current method of fossil fuel combustion. This can not only serve as a mitigation to carbon dioxide emissions but also a reduction to the greenhouse gas effect and global warming. It uses non-pathogenic, sustainable, and disposal materials used in this research, such as stevia, wastewater, or food waste. Integrating eco-friendly materials as renewable energy development is quintessential. This research aims to explore the feasibility of utilizing microbial fuel cells (MFCs) in this manner.

2.Materials and Methodology

2.1.Materials:

- two small containers
- A voltmeter
- Nafion
- Saltwater
- Graphite rod
- Glass knife
- Parafilm (to secure the open ports of the glass container)
- four different microorganisms (Shewanella, Geobacter, Desulfovibrio, Stevia)
- three different organic matter (wastewater, food waste, lignocellulosic material, water)
- refrigerator
- water bath
- plant chamber
- UV lamp

Figure 2. Visual showing the materials involved with experiment 1

Figure 3. Visual showing experiment 1 being conducted

2.2.Experiment 1:

1) Connect the two containers with a Nafion, using a clipper to stabilize
2) Seal the holes in the container with parafilm (2 on each side)
3) Designate the left container as the anode and the right as the cathode
4) Use a glass knife to unbox the microorganisms
5) Put in 120mL of organic matter (wastewater, food waste, lignocellulosic materials) and mix it with the microorganism (Shewanella, Geobacter, Desulfovibrio, Stevia) in the anode chamber
6) Put in 120mL of salt water in the cathode chamber
7) Use a graphite rod to connect the voltmeter to both chambers and observe the electrical charge over the next 72 hours

This experiment used the microorganisms of Shewanella, Geobacter, Desulfoviborio, and Stevia fermentation liquid. Shewanella, Geobacter, and Desulfoviborio serve as iron-reducing bacteria, while Stevia fermentation liquid was used for its abundance in microorganisms. In the anode chamber, as iron-reducing bacteria, Shewanella and Geobacter initiated the oxidation of organic compounds, releasing electrons and protons.

Iron-reducing bacteria play a crucial role in MFCs by facilitating electron transfer processes. These bacteria can transfer electrons to insoluble electron acceptors, which enhances electron flow within the microbial community [4]. In addition to this, iron-reducing bacteria act as biocatalysts in MFCs. Their metabolic activities serve as essential components for the electrochemical reactions within the microbial community, promoting efficient energy conversion.

2.3.Experiment 2:

1) Based on the following three factors, expose the MFC to constant conditions: temperature, precipitation, UV light exposure
2) Set the temperature (˚C) as:
    a) 4˚C, using a refrigerator with consistent temperature
    b) 15˚C, using a water bath to control its temperature
    c) 25˚C, leaving it at room temperature
    d) 36˚C, using a water bath to control its temperature
    e) 60˚C, using a water bath to control its temperature
3) Using a plant chamber, set the precipitation (%) as:
    a) 30%
    b) 50%
    c) 70%
    d) 100%
4) Using a UV lamp (with 395 nanometers), expose the MFC to UV light (watts):
    a) 0 watts
    b) 10 watts
    c) 20 watts
    d) 30 watts

2.4.Experiment 3:

1) Based on the best combinations and conditions explored in experiments 1 & 2, study the long-term effects of 7 days
2) Record the electricity by the hour, monitoring for each group (stevia and wastewater at 25˚C and no exposure to UV light as group 1, stevia and
wastewater at 25˚C with 10-watt UV exposureas group 2, and Shewanella and wastewater at 25˚C and no exposure to UV light as group 3)

3.Result

1. Experiment 1

Figure 4. Visual showing experiment 1 completed

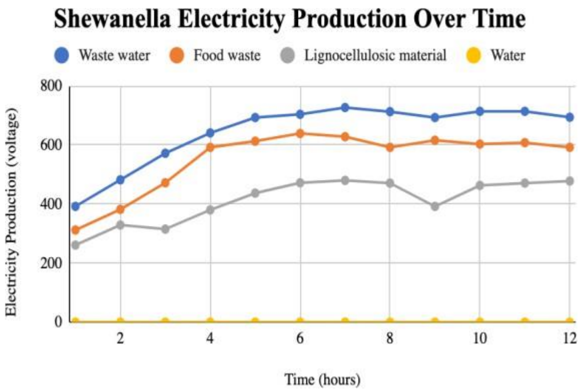

Figure 5. Line graph showing Shewanella electricity production over time with different organic matter

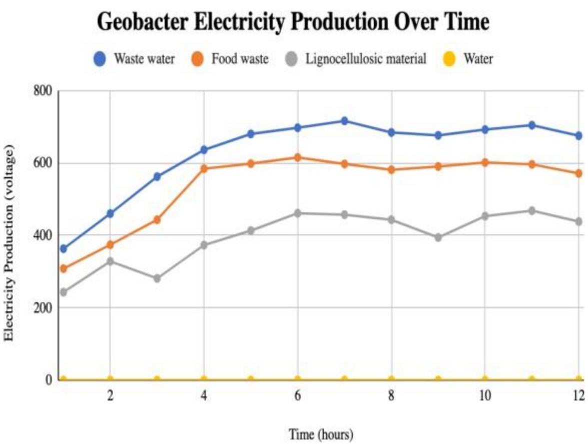

Figure 6. Line graph showing Geobacter electricity production over time with different organic matter

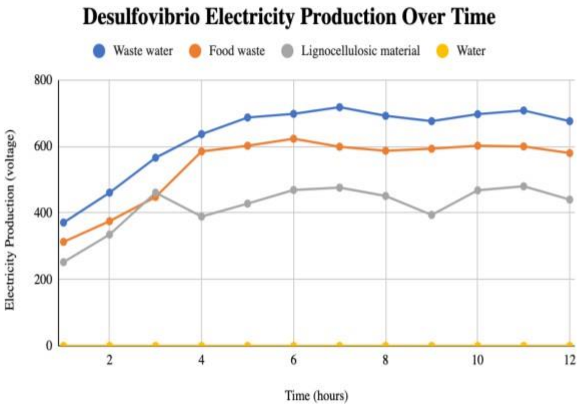

Figure 7. Line graph showing Desulfovibrio electricity production over time with different organic matter

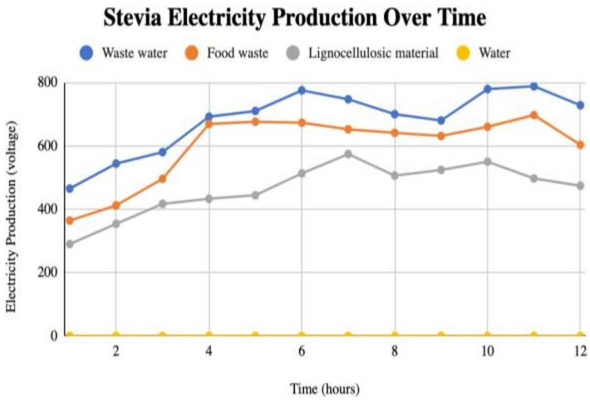

Figure 8: line graph showing Stevia electricity production over time with different organic matter

Table 1. Raw data table showing electricity production (voltage) for Stevia and other organic matter

	Electricity Production(Voltage)												
Hour	0	1	2	3	4	5	6	7	8	9	10	11	12
Waste Water	412	465	544	580	692	710	775	747	700	680	779	788	728
Food Waste	311	364	412	496	669	676	673	652	641	631	660	697	603
Ligno-cellulo-sic	195	290	354	417	433	444	513	574	506	524	550	497	474
water	0	0	0	0	0	0	0	0	0	0	0	0	0

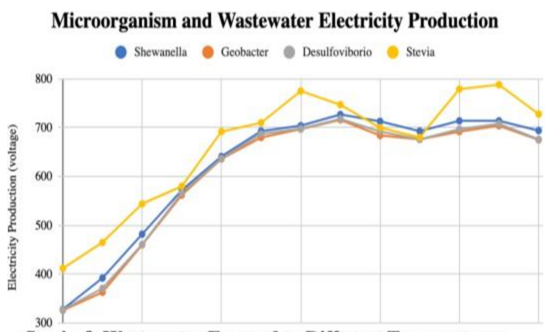

Figure 10. Line graph showing wastewater electricity production over time with different microorganisms

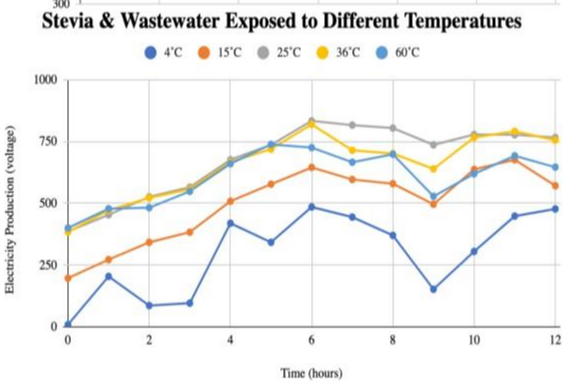

Figure 11. Line graph showing Stevia & wastewater electricity production over time with exposure to different temperatures

The objective of experiment 1 was to find the most energy-efficient combination of microorganisms and organic matter. Out of the 16 different combinations, stevia fermentation liquid and waste water exhibited the largest amount of electricity produced. As opposed to the traditional use of Shewanella in MFCs, stevia fermentation liquid poses a strong alternative for MFC electricity production. The data showed a general increasing trendline in electricity even after 12 hours of initial reaction. The highest voltage measured occurred at 788 volts 11 hours after the initial reaction between stevia and wastewater.

In terms of which organic matter was most effective, the combination of a microorganism + wastewater produced the most electricity consistently throughout the experiment. Apart from stevia, wastewater showed an increasing trend until 5 hours after initial exposure, yet steadily remained at a constant voltage over the next 7 hours measured.

The order in which organic matter produced the most electricity relative to the others was consistent throughout all trials, with wastewater being the most efficient, food waste being the second, lignocellulosic material being third, and water being last. Water could be observed as an anomaly as it produced no electricity. (reason to be explained in the discussions)

2. Experiment 2

The aim of experiment 2 was to find the best condition in which the MFC produced the most electricity by exposing it to various conditions. The best condition in which stevia fermentation liquid and wastewater reaction was found to be at 25˚C, approximately at room temperature. The average volts for this condition was 680 across the 12 hours, with the maximum occurring 6 hours after initial exposure at 833 volts.

For exposure to UV light, 0 and 10 watts were the most successful. As seen in the graph below, there was a constant turnover each hour for electricity produced. The maximum of the 2 occurred 10 hours for 10 watts, with the MFC producing 807 volts.

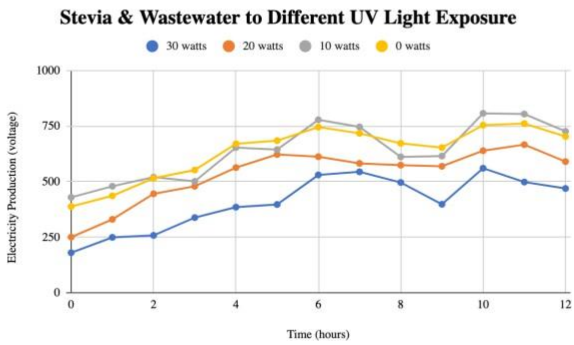

Figure 12. Line graph showing Stevia & wastewater electricity production over time with exposure to different UV light

A significant result was that the temperature condition of 25˚C was closely followed by high precipitation levels. The data revealed that 30%, 50%, 70%, and 100% precipitation conditions showed average volts of 668, 661, 663, and 676, respectively. The voltage differences were not substantial, as mentioned in the discussion below.

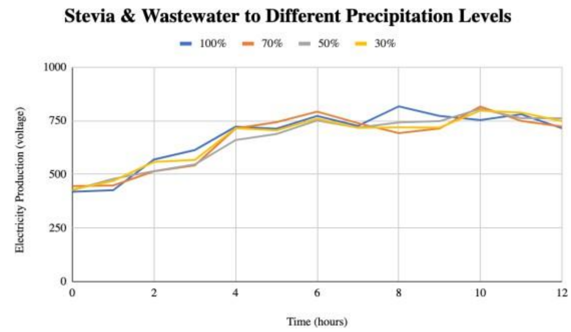

Figure 13. Line graph showing Stevia & wastewater electricity production over time with exposure to different precipitation levels

3. Experiment 3

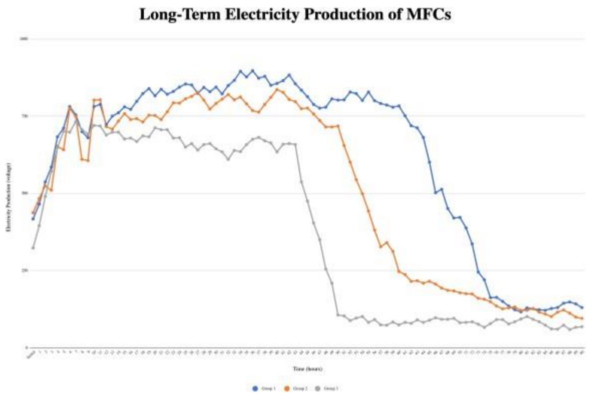

Figure 14. Line graph showing long-term electricity production of different combinations and conditions of MFCs

The data from the experiment showed that the combination of stevia and wastewater at 25˚C and no exposure to UV light proved to be the most ideal condition in MFC electricity production. This experiment aimed to determine the long-term impacts and electricity production. As shown in the graph above, stevia and wastewater at 25˚C and no exposure to UV light (group 1) were consistently higher than the other conditions of stevia and wastewater at 25˚C with 10-watt UV exposure (group 2) or shewanella and wastewater at 25˚C and no exposure to UV light (group 3). This is evidenced by the data shown in the table below, in which the combination of stevia and wastewater at 25˚C and no exposure to UV light had 55 hours of over 80% of the maximum, as opposed to 43 and 40 for stevia and wastewater at 25˚C with 10 watt UV exposure, and Shewanella and wastewater at 25˚C and no exposure to UV light, respectively. This also aligns with the fact that the data showed similar voltage measurements compared to its maximum for all three groups before it underwent a significant fall in electricity. Group 1 had the highest with 60 hours, while groups 2 and 3 showed a significant decrease after 50 and 43, respectively.

Table 2. Raw data table showing the number of hours electricity produced was over 80% of the maximum

	Several hours of electricity produced was over 80% of the maximum.
Stevia and wastewater at 25 ˚C with 0 UV light	55
Stevia and wastewater at 25 ˚C with 10 watts of UV light	43
Shewanella and wastewater at 25 ˚C with 0 UV light	40

4.Discussion

The results obtained from Experiment 1 provided emphasis on the significance of the right combination of organic matter and microorganisms. As demonstrated in the experiments, Stevia's electricity efficiency can be attributed to its unique combination of abundant microorganisms and potent antioxidant properties.

Stevia is primarily known for its sweetening compounds, such as stevioside and rebaudioside. Microbial communities in any given environment, including within plant materials, can vary widely. Stevia fermentation liquid contains diverse microorganisms, including bacteria, yeast, and fungi. By breaking down organic matter and producing byproducts, these microorganisms may contribute to the fermentation process, including electricity in the context of MFCs.

The rich polyphenol content in Stevia contributes to enhanced microbial activities in the anode chamber, fostering increased electron production [5]. The polyphenol content can act as electron shuttles or mediators in microbial fuel cell reactions. These compounds may facilitate electron transfer, enhancing the efficiency of the electricity generation process. Antioxidants can also protect microbial cells from oxidative stress, promoting longevity and activity [6].

While stevia itself is not agar, which is a gelatinous substance derived from seaweed, it may provide a supportive environment for microbial growth in a manner similar to agar. Agar is commonly used in microbiology as a solidifying agent for culture media. It provides a stable and nutrient-rich matrix for microorganisms to proliferate and form colonies [7]. Similarly, the liquid or extract from stevia fermentation could offer a conducive environment for the growth of microorganisms, supporting their metabolic activity in microbial fuel cell experiments.

It could be concluded that room temperature conditions (25, 36˚C) were ideal for electricity production in MFCs. Low exposure was ideal for UV light, with 0 and 10 watts presenting as the most efficient electricity producers. In particular, no exposure to UV light revealed the best results. This can partly be explained by the potential influence of UV metagenesis on DNA within the microbial community. UV light is known to induce genetic mutations through processes like UV metagenesis, which can alter the DNA structure of microorganisms [8]. As a result, this may impact the efficiency of microbial fuel cells by affecting the electrogenic capabilities of the microorganisms involved.

The sensitivity of microorganisms to UV-induced DNA changes may disrupt their metabolic processes and electron transfer mechanisms, leading to a decline in electricity production. The observed efficiency in MFCs under low UV exposure conditions suggests that minimizing UV-induced genetic alterations may be conducive to maintaining optimal microbial functionality and, consequently, electricity generation in microbial fuel cells.

However, unlike the 25˚C condition, every precipitation condition presented high electricity values, potentially signifying insignificance.

It is also worth noting that the data showed that electricity in voltage maintained a fairly constant voltage after a significant drop for all three groups, with the voltage staying around 100-200 for both groups 1 and 2. Such also occurred for group 3, with all its data points between 80-100. This poses another point of further research, as the electricity does not entirely deplete after a certain period.

5. Conclusion

The research concentrated on investigating Microbial Fuel Cells (MFCs) as a sustainable alternative to conventional energy sources, with a specific focus on addressing the environmental repercussions associated with the combustion of fossil fuels. Throughout the study, the researchers explored the potential of Stevia fermentation liquid as an organic material for MFCs.

The method used in this research yielded several strengths: it identified an unexplored combination of Stevia fermentation liquid and wastewater, shedding light on its potential for electricity production within MFCs. Particularly compared to the traditional control group, Shewanella showed prominent results and could emerge as an alternative soon. The research also delved into the long-term effects of the identified combination, highlighting its consistent and sustained electricity production. Additionally, the combination study found that the combination of Stevia and wastewater proved to be non-pathogenic as opposed to pathogenic microorganisms such as Shewanella, addressing environmental and human safety concerns. Furthermore, the research considered various environmental variables, including UV exposure, temperature, and precipitation, providing several points of comparison that aided the understanding of their impact on MFC performance.

However, the research did possess limitations: the study focused on a limited set of microorganisms, involving only four types. This limitation underscores the need for a more diverse exploration, particularly in the context of iron-reducing bacteria. Availability constraints posed challenges, as some bacteria could not be included due to issues related to their accessibility. The study also conducted only a single experiment without engaging in multiple trials, potentially reducing the robustness of the findings, which occurred due to time constraints and the author’s student status.

The research suggests avenues for further exploration, such as accurately identifying microorganisms in Stevia fermentation liquid, which may necessitate applying advanced techniques such as metagenomic analysis, microbial isolation, or 16S rRNA gene sequencing. Additionally, a more detailed exploration of Stevia is needed, considering that existing studies have primarily focused on its phytochemical composition rather than the specific microorganisms within its fermentation liquid, which could significantly change future results of electricity production of MFCs.

The potential implications are also highly significant. If MFCs prove to be a reliable substitute for the current fossil fuel-based resources, they have the potential to establish themselves as a novel and eco-friendly energy source. This result is in harmony with both economic and environmentally conscious practices, as the study employed materials designated for disposal with easy accessibility, such as wastewater or Stevia, to generate electricity, illustrating a tangible application of sustainability principles.

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