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RESEARCH ARTICLE
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ISOLATION OF ELECTROGENIC BACTERIA AND THEIR POTENTIAL FOR SUSTAINABLE ENERGY PRODUCTION IN MICROBIAL FUEL

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ABSTRACT

Despite their nasty reputation, many varieties of bacteria contribute significantly to our lives, from helping to clean up the environment to improving our health. They have the power to affect our emotions and motivate us to seek food, which can lead to feelings of grief, joy, and hunger. Bacteria also help the brain and gut communicate with one another. Some useful bacteria can be used to solve several problems, including waste management and pollution reduction. Surprisingly, certain bacteria are even electrogenic and possess special skills. Energy is essential to the survival of all living things on Earth, including humans. Microbial fuel cells' (MFCs) function depends on exoelectrogenic bacterial species, which aid in extracellular electron transport. Microbial fuel cells (MFCs) are a sustainable renewable energy technique that uses bacteria to oxidize organic or inorganic substrates, resulting in electrical energy. Several exo-electrogenic bacterial species, recognized for their ability to generate significant electricity in MFCs, have been found to use a variety of organic molecules as fuel. This study investigates the energy generation capabilities of the two mixed & isolated pure strains of bacteria extracted from rice paddy field soil. The study involves extracting exoelectrogenic bacterial species from soil samples, measuring bacterial growth using the measurements of optical density (OD), cell dry weight (CDW), and viable cell count, and calculating the electricity generation. A mixed bacterial culture from paddy field soil produced 77.62 µW of peak power and 0.70 mA of current, This higher performance can be due to the synergistic connections between diverse bacterial species in this mixed culture, which likely promoted more efficient electron transport and better substrate breakdown whereas a pure bacterial strain produced 51.32 µW and 0.28 mA. This study

highlights the benefits of utilizing mixed microbial cultures in MFCs, which improve electricity output while maintaining stability over time.

Keywords: Electrogenic bacteria, microbial fuel cell, cabel bacteria, microbial nano wires, shewanella, geobacter, bacterial batteries, bio energy

INTRODUCTION

All life forms on Earth – even humans – must harness energy if they are to remain alive. This energy comes in the form of electrons, the same tiny negatively charged particles that create a current when they zip around electrical wires in a circuit. We humans, along with most other organisms on this planet, get our electrons from sugars in the food that we eat. In a series of chemical reactions that happen inside our cells, the electrons are released and ultimately flow into oxygen – the same oxygen that we have just breathed in through our lungs. That flow of electrons is what powers our bodies. Similarly, all other living cells are also ultimately powered by electrons. Most species get electrons from food, but some bacteria can survive on nothing but pure electricity. This means that the challenge for all creatures is the same. Whether the organism is a single-celled bacterium or a blue whale, it has to find a source of electrons and a place to dump them to complete the circuit. Some microbes have developed the ultimate stripped-down diet. They do not bother with food or oxygen. All they need to survive is pure electrical energy[1].Microbial fuel cells' (MFCs) function depends on exoelectrogenic bacterial species, which aid in extracellular electron transport. Microbial fuel cells (MFCs) are a sustainable renewable energy technique that uses bacteria to oxidize organic or inorganic substrates, resulting in electrical energy. Several exo-electrogenic bacterial species, recognized for their ability to generate significant electricity in MFCs, have been found to use a variety of organic molecules as fuel [2].

The microbial fuel cell (MFC) has gained much attention because of its ability to generate power from organic or inorganic compounds via microorganisms. Around one hundred years ago, the technology of generating electricity through bacteria was found But it did not gain much attention. Due to the ability to convert chemical energy to electrical energy, MFCs have many potential applications, such as electricity generation, bio-hydrogen production, wastewater treatment, and biosensors. . MFCs became more attractive in real applications, for instance, wastewater treatment and power generation[4]. Microorganisms oxidize substrates in the anodic chamber to produce electrons and protons while producing carbon dioxide as an oxidation product. Electrons attached to the anode (negative terminal) flow to the cathode (positive terminal) through an external circuit. Protons migrate across the proton/cation exchange membrane to combine with electrons to form water if oxygen is provided [5] Or to form ferrocyanide if ferricyanide is provided. Therefore, a positive current flows from the positive terminal to the negative terminal, and this direction is opposite to the electron flow [6]. This is how MFCs generate electricity through microorganisms. Fuel cells (such as batteries) generate electricity by separating the electron donor (anode) from the electron acceptor (cathode) [7]. Microbial fuel cell (MFC) uses electrochemically active bacteria (EAB) as biocatalysts to convert biodegradable waste into electricity [8]. EABs are gaining importance because of their electron-donating ability to the electrodes in MFC. Microorganisms such as members of the *Geobacter* family, *Shewanella putrefaciens* and *Shewanella oneidensis*, *Rhodoferax ferrireducens*, *Pseudomonas aeruginosa*, *Clostridium butyrium*, and *Aeromonas hydrophila* have been reported to oxidize organic matter at the anode to complete their metabolism process. Despite being a promising technology, MFC suffers from limitations such as low power density, high cost, etc. MFCs will be a viable option for power generation if the current production of this device is improved [9]. The electrochemical performance of the bio-anode is one of the major factors that affect the bioelectricity generation process therefore identification of key operational factors and their optimization is of utmost importance for the maximization of the power output [10]. Electrode modification is another strategy to improve the performance of an MFC [11]. Thus the present study endeavored in this direction and was focused on improving power generation using *Shewanella sp.* as an anodic biocatalyst in a single-chambered MFC (sMFC)[12].

Microbial fuel cells (MFCs) harness the electrons generated by bacteria (for respiration) to power fuel cells. Specifically, the microbial biofilms are grown on the anode where they separate hydrogens from the substances provided to them as food (microbial food can include wastewater, acetate, formaldehyde, etc.). To reach the cathode (spontaneous reaction), electrons must travel through the resistor, and the protons left behind move to the cathode through a proton exchange membrane. At the cathode, oxygen can act as a terminal electron acceptor, and the electrons and protons are combined with oxygen to make water [13].

Along with the understanding of the MFC concept, many MFC-based applications have emerged, such as wastewater treatment, microbial electrolysis cells, sediment MFCs, and bioremediation. Several MFC applications will be explained in this section. Among those MFC-based technologies, the most immediate and useful one is as a method of wastewater treatment [14]. The electricity produced by MFCs can be used for powering other technologies, such as biologically inspired robots, some small devices, or remote devices. In addition, the voltage generated by MFCs can be used on microbial electrolysis cells (MECs), which is a modified MFC-based system to produce H_2/ H_2O_2 instead of electricity [15].

Electric bacteria have a lot of applications concerning their uses, by keeping their applications in mind we can get a lot of advantages from electric bacteria whether it is *shewanella*, *geobacter*, or any type of electric bacteria. If we talk about the dark fermentation of electric bacteria which produces bio-hydrogen (important because of environmentally friendly and energy-intensive). Microorganisms found in marine sediment, soil, freshwater sediment, or activated sludge like *Geobacter*, *Shewanella*, Pseudomonas, and Clostridium are responsible for the generation of electricity through fuel cells known as MFC. The development of MFC that can harvest electricity from the organic matter in aquatic sediments is another emerging application of electric bacteria. Other exciting applications include sophisticated nanomachines, biosensors, bioenergy, bioremediation, bio-electronics, potential targets for pathogenic micro-organisms, biofuel, and bacterial batteries[16].

It seems that bacteria have been plugged into each other all along and this finding could open entirely new opportunities in bioenergy production.

METHODOLOGY

1. MFC Configuration

A single-chamber MFC with a graphite thread felt anode and cathode that were connected by titanium wires was used. The MFC vessel could accommodate 400 mL of water feed & was designed to reduce oxygen intrusion near the anode and still provide an anaerobic atmosphere for microbial growth[17].

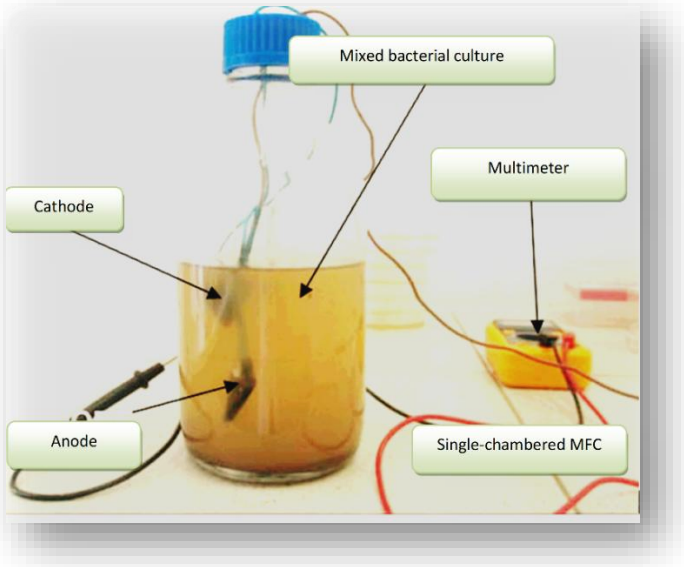

Figure 1: single-chambered MFC.

2. Sample Collection and inoculum Preparation

Soil samples were taken from rice paddy farms. To avoid contamination, the soil, which served as both substrates & bacterial inoculum was collected sterilely. To isolate bacteria from the soil, sterilizing paddy soil was reinoculated with strains following incubation in liquid broth. This ensured adequate bacterial concentration for electricity generation.

3. Electricity Generation and Resistance Application

The MudWatt Exploration app was used to monitor electricity generation, recording current and voltage values at regular intervals[18]. The power output was measured using resistors ranging from 47 to 4700 Ω. Dimensions were occupied when the LED blinking stabilized [19].

4. Isolation of Exoelectrogenic Bacteria from MFC anode surface

To isolate microorganisms developing on anode surfaces, the electrode's surface was cleaned with a jet of sterilized water until any apparent debris particles were eliminated[20]. The first millimeter from the graphite electrode (the anode in the sludge) was vigorously scrapped off using a sterile razor blade in 1.5 mL of the phosphate buffer (50 mM) at a pH of roughly 7.2, yielding a suspension of graphite with electrode-associated microorganisms. The resulting solution was serially diluted to 10-6 and plated on Mueller-Hilton (MH) agar plates [21].

5. Morphological Characterization

Following the incubation time, morphologically different colonies were removed from the Petri plates and then restacked in suitable media to obtain pure cultures. Bacterial strains have been collected, cultured, and preserved on MH agar. Bacterial forms vary enormously. Adaptive forces that enhance bacterial fitness result in certain morphologies. Shape influences significant biological processes that include nutrition intake, motility, dispersal, resilience to stress, or interspecies interactions. Gram stains were used according to instructions by Merchant & Packer[22] to identify the dimension, form, and location of those bacteria.

6. Monitoring *Bacterial* growth

Optical Density (OD600) Bacterial culture growth was measured spectrophotometrically by measuring optical density (OD600) periodically over 7 days [22].

Cell Dry Weight (CDW): The bacterial biomass was evaluated by centrifuging the suspension of the bacterium and weighing the dried residue[23].

CDW was calculated as:

$$\mathbf{CDW(g/mL) = \frac{M2-M1}{1mL}}$$

Viable Cell Count: The amount of live bacterial cells was counted utilizing a hemocytometer & trypan blue staining.

7. Preparing a Bacterial Suspension

To make certain that the bacterial cells were distributed evenly, the culture of bacteria in the tube was gently swirled.100 µL of the suspended bacteria was moved into a sterile microcentrifuge chamber using a sterile pipette. The identical microcentrifuge tube was immediately filled with 100 µL of trypan blue solution. To ensure even mixing, the mixture was pipetted up and down several times to combine it well[24].

8. Using a Compound Light Microscope to View

A suitable volume of the suspension was cautiously moved to a hemocytometer after mixing. Through capillary action, the suspension was permitted to permeate beneath the cover slip (28). The hemocytometer was examined at 40x magnification using a compound light microscope. Five chosen

squares of the hemocytometer grid were counted to determine the total number of cells. The following formula was used to get the viable cell count:

$$\text{Viable Cell Count} = \frac{\Sigma \text{ (cells in five squares)} \times 1000 \times \text{dilution factor}}{0.004\ mm^3}$$

9. Soil Inoculation with Exoelectrogenic Bacteria Species

To get rid of undesired microbiological contamination, the soil sample was sterilized twice at 48-hour intervals using 121°C for 30 minutes each time. To ensure total sterilization, UV light was applied to the MFC chamber. After being cultivated in nutritious broth for 8–16 hours, the pure bacterial culture was chosen for inoculation to make sure it reached its exponential growth phase. A spatula was used to evenly mix 8.6 mL of the produced bacterial culture with the sterilized soil. After that, the inoculated soil was cautiously put into the MFC chamber for additional testing[25].

10. Monitoring the performance and generation of electricity

To determine the MFC's power output, external resistors were used to measure current and voltage. Maximum power production and current were measured at various times, and the inner resistance was determined[26].

The study evaluated electricity generation amongst mixed bacterial cultures as well as pure bacterial isolates. The performance was measured as a result of peak power output, current, and voltage**.**

RESULTS

1. Morphological and Microscopic Analysis of Selected Strains

The acquired pure bacterial cultures were then cultivated on Mueller-Hilton agar and phenotypically characterized. Strain A1 colonies were noted to be opaque, dark yellow, seamless, and shining when grown in a nutrient medium. Strain A1 bacteria are Gram-positive, long rods in structure. Strain A2 colonies have a fluffy white or light-yellow hue, and they are opaque, spherical, rough, and jagged. The bacteria in strain A2 include Gram-positive bacilli featuring blunt ends with oval terminal spores**.**

Figure 2: pure culture of isolates.

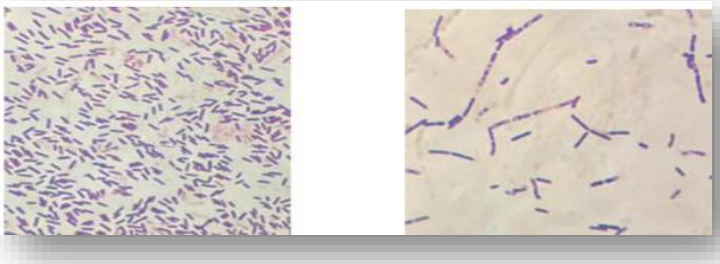

Figure 3: gram staining of isolates

2. Bacterial growth analysis

Bacterial growth analysis revealed a conventional growing curve, with an exponential phase found within the first 8 hours. The maximum optical density (OD600) was detected on day 6, followed by a fall on day 7, showing that bacterial growth has entered the dying phase. The bacterial time needed for doubling was calculated as 2.9 hours, which is comparable with the growth properties of many electrogenic bacteria shown in **(Fig 4,5 & 6).**

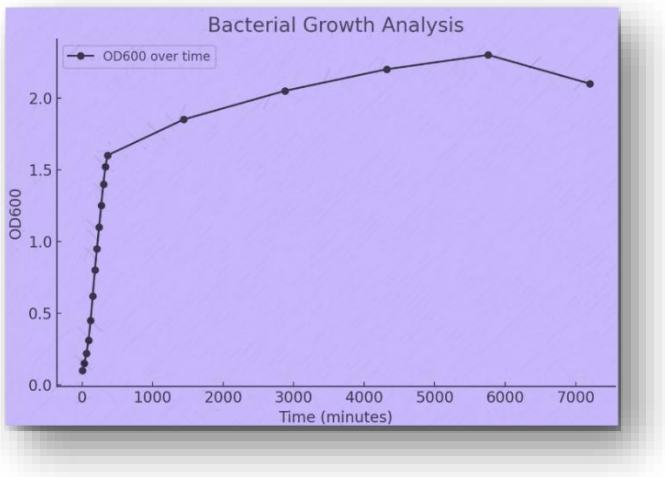

Figure 4: The bacterial growth analysis graph shows OD600 over time

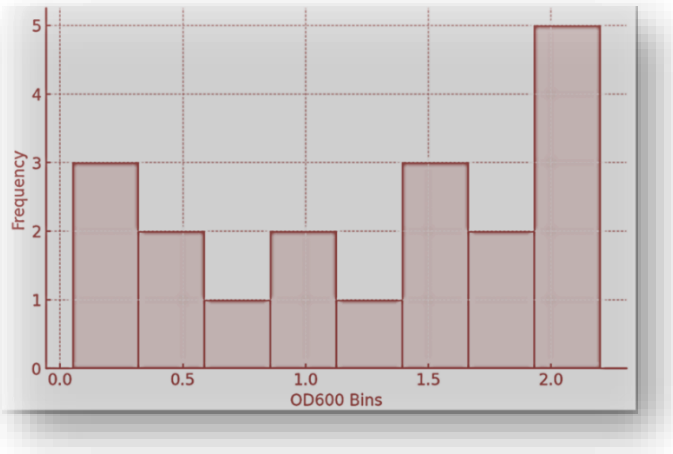

Figure 5: depicts the spread of bacterial growth metrics. The x-axis depicts the OD600 values separated into bins, with the y-axis shows the average amount of occurrence across every range.

Explanation

The graph depicts bacterial growth over time by calculating optical density at 600 nm (OD600), which represents cell density in the culture. The curve shows an ordinary bacterial growthtrend.
1. During the lag phase, bacteria gradually raise their OD600 levels as they adjust to their newhabitat.
2. During the exponential phase, there is a significant increase in OD600 within the very first 8 hours (~480 minutes), reflecting rapid bacterial division. The predicted doubling time was 2.9 hours, which is consistent with the growth properties utilized by numerous electrogenic bacteria.
3. Stationary Phase: Development slows and plateaus, indicating nutritional limitations and waste accumulation.
4. The OD600 begins to decrease on day 7 (~7000 minutes), indicating bacterial cell death owing to resource depletion and hazardous byproduct accumulation.

On day 6 (~6000 minutes), the greatest OD600 value was measured, indicating the pinnacle of the development of bacteria before death.

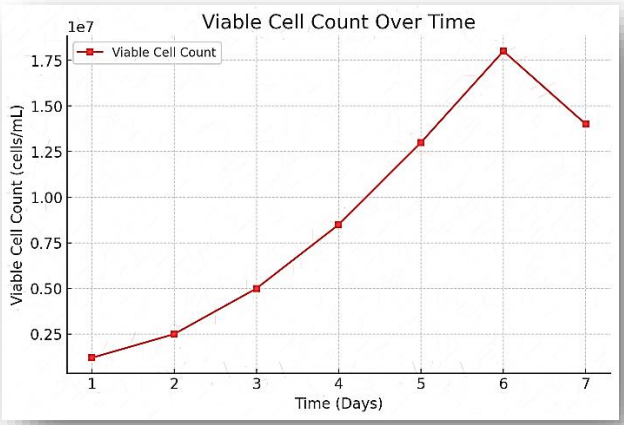

Figure 6: viable cell count graph over time (Day 1 to Day 7).

Fig 6 shows the Bacterial growth phases indicated by the graph, which displays the viable cell count for seven days. The exponential growth phase is indicated by the count rising from 1.2×10^6 cells/mL (Day 1) to a peak of 1.8×10^7 cells/mL (Day 6). The count drops to 1.4×10^7 cells/mL on Day 7, signaling the start of the dying phase brought on by nutritional shortage. Day 6 is the ideal bacterial growth point for MFC applications, as this pattern is consistent with OD600 data.

3. Generating electricity

Mixed Culture Performance

On day 14, the MFC using mixed bacterial cultures using rice paddy field soil had a peak power production of 77.62 µW with a current of 0.70 mA, outperforming pure cultures. The mixed culture MFC produced a more stable and constant power output over time, most likely due to syntrophic interactions between several bacterial species.

Pure Culture Performance

In contrast, the MFC infected with a purified bacterial strain had a peak power production of 51.32 µW with a current of 0.28 mA on day 13. The pure strain produced less power and was less stable than the mixed culture shown in **(Fig 7& 8)**.

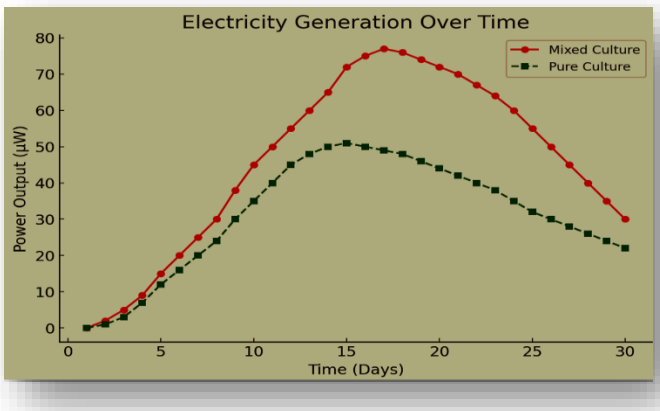

Figure 7: electricity generation comparison graph, showing power output over 30 days for mixed and pure cultures

Explanation

In above **Fig 7** graph depicts energy generation time passes, in a microbial fuel cell (MFC) utilizing two distinct bacterial cultures: a hybrid culture and a pure culture.

Mixed Cultural Performance (Red Line with Circles)

The MFC containing mixed cultures of bacteria from rice paddy soil performed well. On day 14, it had the highest power consumption of 77.62 µW and an average current of 0.70 mA. The power output became more stable and constant over time, probably because of syntrophic interactions among different bacterial species. After reaching its peak, power generation progressively dropped.

Pure Cultures Performance (green line connecting squares)

On day 13, the MFC infected with a pure bacterial strain achieved a maximum output of electricity of 51.32 µW and a current of 0.28 mA. When compared to mixed cultures, the electricity output was lower and less stable. Power output decreased after peaking, although it maintained lower than the mixed culture constantly.

This study emphasizes the benefits of utilizing mixed microbial cultures in MFCs, which improve electricity output while maintaining stability over time.

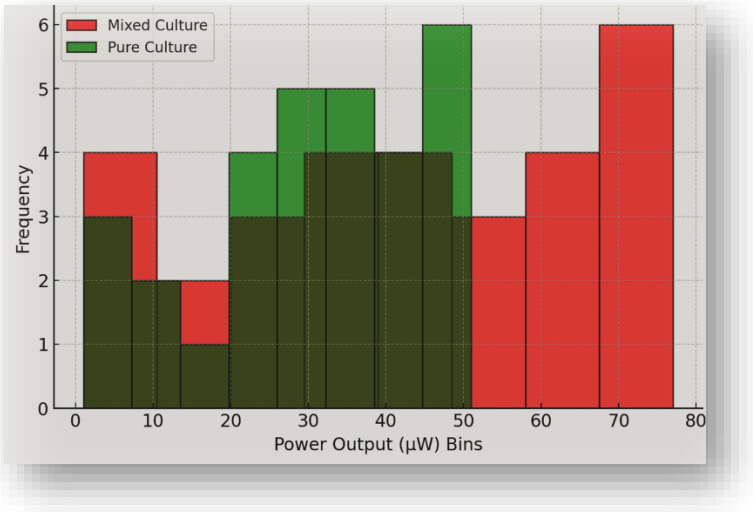

Figure 8: The power output dispersion for mixed with pure bacterial cultures with the MFC.

Explanation

In Figure 8 The power output dispersion for mixed pure bacterial cultures with the MFC is shown below:

Red bars show the mixed culture has a wider spread and greater power output values

Green bars show the pure culture, which has a concentrated range of lower power outputs.

The mixed culture has a wider spread and greater peak values, whereas the pure cultures are more restricted in power output. This histogram clearly emphasizes the beneficial effects of combination cultures in microbial fuel cells in terms of generating greater quantities and more predictable electrical power over time.

3. maximum Power and Current Output

The mixed culture MFC had an internal resistance of 470 Ω, whereas the pure culture MFC had a resistance of 2200 Ω. The mixed culture's lower internal resistance indicates improved electron transfer effectiveness and conductivity, which is most likely owing to collaborative interactions among several microbial species shown in **(Fig 9 & 10).**

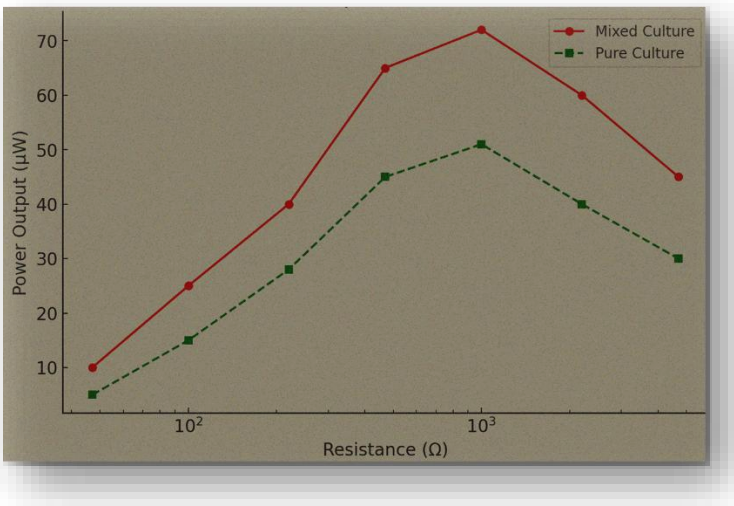

Figure 9: The power output vs. resistance graph shows how power varies with different resistor values for mixed and pure cultures

Explanation
Using both mixed & pure bacterial cultures, this graph shows the link between resistance and power production for microbial fuel cells (MFCs).

Internal Resistance Differences:
A lower interior resistance in a mixed culture indicates superior conductivity and electron transfer efficiency. The purest culture MFC had a higher internal resistance of 2200 Ω, whereas the mixed culture MFC had 470 Ω.

Mixed Culture (Red Line with Circles)
increased power production at all resistance levels.
At an ideal resistance, it peaked at about 72 µW before starting to drop.

Pure Culture (Green Line with Squares):
Produced less power, reaching a maximum of about 50 µW when the resistance was higher.
Extreme resistance resulted in a reduced power output that decreased faster.

Figure 10: how power output values from both mixed culture & pure culture MFCs are distributed across various resistances.

Explanation
This histogram shows how power output values from both mixed culture & pure culture MFCs are distributed across various resistances.

Mixed Culture (Red Bars)
Its superior electron transfer efficiency is further supported by a wider distribution, with power output peaking at 72 µW and more frequent frequencies in the mid-to-high range.

Pure Culture (Green Bars)
The distribution is narrower, suggesting less electron transfer efficiency, as well as more concentrated with lower power output ranges, peaking at 50 µW. This further supports the discovery that mixed cultures perform better in MFCs than pure cultures because of improved syntrophic interactions and lower internal resistance.

Syntrophic Interactions
Because of syntrophic interactions, which occur when various bacterial species cooperate to maximize the breakdown of organic substrates and increase electron transfer efficiency, mixed cultures exhibit increased power output. Because they offer alternate metabolic pathways for the breakdown of organic matter, mixed microbial consortia have been demonstrated to improve electron transfer in MFCs.

4. DISCUSSION

The study's findings highlight how important microbial diversity is to improving the production of electricity in microbial fuel cells (MFCs). In contrast to pure bacterial isolates, which produced 51.32 µW and 0.28 mA, mixed bacterial cultures of rice paddy field soil achieved a peak power production of 77.62 µW with a current of 0.70 mA. This higher performance can be due to the synergistic connections between diverse bacterial species in this mixed culture, which likely promoted more efficient electron transport and better substrate breakdown. These bacteria varied metabolic capacities enable them to use available substrates more efficiently, which eventually results in higher and more consistent power outputs. Microbial consortia can improve the conductivity & electron transfer efficiency within MFCs, which is crucial for maximizing power generation, as evidenced by the mixed culture MFC's lower internal resistance (470 Ω) in comparison to the pure culture MFC (2200 Ω).
While individual species like *Geobacter* and *Shewanella* are well-established for their exoelectrogenic properties, mixed cultures can leverage the complementary metabolic pathways of various species to achieve superior overall performance. These findings are consistent with previous research that has demonstrated that mixed microbial communities frequently outperform pure bacterial cultures in terms of power output and stability in MFCs. For example, in a study employing *Geobacter sulfurreducens* along with *Shewanella oneidensis* in MFCs with a substance called the substrate, the performance of these isolated electrogenic bacteria was significant, but the peak power output did not surpass that of the mixed cultures in our study [26].
The findings are also in line with another study on the production of electricity from soil bacteria in paddy fields, which showed that combined bacterial consortia generated better electricity because different bacteria played complementary roles in electron transfer and substrate degradation[23]. The belief that microbial diversity can promote a more robust and efficient bioelectricity generation system is further supported by the fact that, in our case, the mixed culture MFC demonstrated more consistent electricity generation throughout the experimental period[27]. These findings underline the necessity of researching complex microbiological communities for MFC applications, rather than relying simply on well-characterized, single-species culture.

5. CONCLUSION

This study demonstrates that bacteria extracted from rice cultivation soil have substantial potential for power generation in MFCs. Mixed bacterial cultures outperformed isolated bacteria in terms of

electrical power output, indicating the relevance of microbial diversity for better electricity production. According to the results, using complicated communities of bacteria in MFCs can result in more reliable and effective power production. Future studies could concentrate on enhancing the production of bioelectricity in microbial fuel cells by designing microbial consortia and optimizing substrate usage.

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