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Electricity Generation by Electrogenic Bacteria using Wastewater as Nutrient

Meaad Ahmed Ayoub Ahmed [1], Ahmed Abass Osman Alhaj [2*]
[1,2] Department of Microbiology and Molecular Biology, Al Neelain University, Khartoum, Sudan

توليد الكهرباء بواسطة البكتيريا المولدة للكهرباء باستخدام مياه الصرف الصحي كمغذيات

ميعاد أحمد أيوب أحمد[1]، أحمد عباس عثمان الحاج[2*]
[1،2] قسم الأحياء الدقيقة وعلم الأحياء الجزيئي، جامعة النيلين، الخرطوم، السودان

*Corresponding author: ahmedalhaj@neelain.edu.sd

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Abstract:
This study focuses on finding and describing bacteria from wastewater that can generate electricity, looking at how well they work without oxygen and how they can be used to clean wastewater. Wastewater specimens, collected from diverse locales in Khartoum state, underwent isolation processes employing serial dilution and plating on LB agar medium, resulting in the identification of bacterial isolates including Actinomyces sp, Bacillus sp, Pseudomonas aeruginosa, Staphylococcus sp, Micrococcus sp, Citrobacter sp, Enterobacter sakazakii, and Enterobacter areogenesa. The isolates were authenticated via biochemical methodologies and selective media. Subsequent assessment of the electrogenic potential of these bacteria, both in pure and mixed cultures, occurred within Microbial Fuel Cells (MFCs) utilizing untreated wastewater. Remarkable electricity generation was observed in the mixed culture at 37°C, yielding 1.2 V. Additionally, Actinomyces sp in the pure culture at 30°C demonstrated significant electrochemical output, generating 353 mV after a five-day incubation period. Concurrently, comprehensive analyses of wastewater quality indicators, encompassing total suspended solids and total dissolved solids, revealed substantial reductions, affirming the adept wastewater treatment capabilities of the isolated bacteria. This study underscores the prospect of leveraging wastewater-derived bacteria for electrochemical applications and wastewater treatment, presenting a resource-efficient and sustainable pattern.

Keywords: Electrogenic Bacteria, Wastewater Remediation, Microbial Fuel Cells (Mfcs), Electrochemical Capabilities.

الملخص
تركز هذه الدراسة على تحديد ووصف البكتيريا المستخرجة من مياه الصرف الصحي التي يمكنها توليد الكهرباء، مع دراسة كفاءتها في العمل بدون أكسجين وكيفية استخدامها في تنظيف مياه الصرف الصحي. تم جمع عينات مياه الصرف الصحي من مواقع مختلفة في ولاية الخرطوم (السودان). تم عزل البكتيريا باستخدام تقنيات التخفيف المتسلسل وزراعتها على وسط آجارLB ، مما أدى إلى تحديد أنواع بكتيرية تشمل الاكتينومايسيتس، العصوية، الزائفة الزنجارية، العنقودية، المكورة الدقيقة، السيتروباكتر، الانتيروباكتر ساكازاكي، والانتيروباكتر ايروجينوسا. تم تأكيد هذه العزلات باستخدام الطرق البيوكيميائية والوسائط الانتقائية. تم تقييم القدرة الكهربية لهذه البكتيريا، سواء في المزارع البكتيرية النقية أو المختلطة، داخل خلايا الوقود الميكروبية (MFCs) باستخدام مياه الصرف غير المعالجة. لوحظ توليد كهرباء ملحوظ في المزارع البكتيرية المختلطة عند 37 درجة مئوية، حيث أنتجت 1.2 فولت. بالإضافة إلى ذلك، أظهرت الاكتينومايسيتس في الثقافة النقية عند 30 درجة مئوية إنتاجًا كهربائيًا كبيرًا، حيث ولدت 353 ملي فولت بعد فترة حضانة لمدة خمسة أيام. في الوقت نفسه، أظهرت تحاليل جودة مياه الصرف انخفاضات كبيرة في المواد الصلبة العالقة الكلية والمواد الصلبة الذائبة الكلية، مما يؤكد فعالية البكتيريا في معالجة مياه الصرف الصحي. تبرز هذه الدراسة إمكانات استخدام البكتيريا المستخرجة من مياه الصرف الصحي في التطبيقات الكهروكيميائية ومعالجة مياه الصرف الصحي، مما يعرض نموذجًا فعالًا ومستدامًا من حيث الموارد.

الكلمات المفتاحية: البكتيريا المولدة للكهرباء، معالجة مياه الصرف الصحي، خلايا الوقود الميكروبية(MFCs) ، القدرات الكهروكيميائية.

Introduction

Excess pressure on the environment and the growing population has led to environmental pollution and the poverty of available resources to produce the endless needs of human beings. Therefore, scientists searched for alternative, inexpensive and ecofriendly ways to cover the human needs and upgrade livelihoods. Life currently depends in all of its aspects on the presence of water and electricity for life. Scientists found the solution in water.as almighty said: (and we made from water every living thing) where they used wastewater to produce electricity, re-clean it to be in use. Just by using supernatural bacteria, bacteria are single celled organisms lacking a distinct nucleus [1].

Wastewater contains chemical energy that can be converted to electrical energy through the respiration of microorganisms that live in wastewater [2]. Electricity is a negative electrical charge carried by electrons; the movement of these negatively charged electrons is called electricity. Electrogenic bacteria are anaerobic and facultative anaerobic bacteria that can transfer electrons to an anode (i.e. as terminal electron acceptor) [3]. In a microbial fuel cell (MFC), electrons are released at the anode, causing the anode to have a higher negative charge, also known as electric potential, than the cathode (Bioenergy Education Initiative). Wastewater is inexpensive, eco-friendly energy source, and report about use wastewater as fuel for generating electricity is few. They are other substrate use to electricity production like carbohydrate [4], textile effluents [5], domestic waste [6, 7], industrial waste [8, 9], agricultural waste [10-12], sea sediments and waste matter in land fill [13].

Materials and Methods

Collection of Samples

Three distinct wastewater samples were aseptically collected in sterile 400 ml containers from disparate water drains in Khartoum, Sudan, in accordance with the Environmental Protection Agency (EPA) guidelines outlined in June 2007. The initial sample was acquired from a water drain proximate to the Jackson tram terminus, the second from a drain adjacent to Khartoum Stadium, and the third from the Al Neelain University manhole. Subsequent to collection, all three samples were promptly preserved at 4°C to maintain the integrity of their microbial and physicochemical characteristics.

Microbial Isolation from Wastewater SedimentsA subsection

Following the amalgamation of three wastewater samples, the combined mixture was allowed to settle for an entire day at 4°C to facilitate sedimentation. The resulting sediment was harvested and subjected to iterative dilutions up to 10-6 using standard saline solution (0.9% NaCl) [14]. Subsequently, 0.1 ml aliquots from each dilution were evenly spread using a spreader onto LB Agar media comprising 0.6 g of yeast extract, 1.2 g of NaCl, 1.2 g of peptone, 2.4 g of agar-agar, and 120 ml of distilled water [3], covering dilutions of 10-1, 10-2, 10-4, and 10-5. Following the Gram stain technique [15], and an incubation period at 30 °C for 24 hours, microscopic examination was conducted to observe distinct colonies. Subsequent verification of Gram stain results was achieved through the application of the KOH test [16].

Identification of Distinct Bacterial Colony

To isolate enterobacteriaceace family that may be found, the isolate that showed Gram- negative were subcultured on EMB Agar. And Gram-positive isolates subcultured on nutrient agar for further identification. Each bacterium was identified by its selective media and biochemical test. The isolated bacteria were *Pseudomonas aeruginosa*, *Citobacter sp*, *Enterobacter sakazakii* and *Eenterobacter aerogenes*. The Gram-positive bacteria after subculture in nutrient agar, they were subcultured many times to reach pure colony and found as *Staphylococcus sp*, *Actinomyces sp*, *Bacillus sp* and *Micrococcus sp*. Then, each one subculture in different agar media for further identification. *Bacillus sp* and *Actinomyces sp* identified by colony morphology and Gram stain, and growth in anaerobic condition. *Staphylococcus sp* and *Micrococcus sp* identified by mannitol salt agar media, oxidase test and anaerobic growth.

Calculation of Total Suspended and Total Dissolved Solid

Determination of Total Suspended Solids in Wastewater Using Modified Whatman Filter Paper Method

In this study, a modified method for the quantification of total suspended solids (TSS) in wastewater (100 milliliters) using Whatman filter paper 0.45 is proposed. The procedure involves two main steps:

the initial assessment of untreated wastewater and a subsequent examination of wastewater treated with bacterial growth. For the untreated wastewater, the filter paper was initially weighed (W1). Subsequently, 100 milliliters of wastewater underwent filtration, followed by drying in a 150°C oven for two hours. The filter paper was then re-weighed, yielding a second weight (W2). This process was replicated for wastewater treated with bacterial growth. After treatment, the filter paper was again weighed (W1), and the filtered wastewater was then weighed (W2) using a sensitive balance. The modified method offers a comprehensive approach, ensuring the accurate determination of total suspended solids in both untreated and treated wastewater samples.

Determination of Total Dissolved Solids (TDS)

The analysis of Total Dissolved Solids (TDS) began with the precise measurement of the lid of a petri plate with a 20 ml capacity. Subsequently, 20 ml of wastewater was introduced into the petri plate, followed by drying in a 150°C oven for two hours, with additional overnight standing. Upon re-weighing the petri plate, the recorded weight was noted. This protocol was replicated for wastewater subjected to bacterial growth treatment. The initial weight of the petri plate post-treatment remained consistent, while the treated wastewater weight was measured with a precision balance. This method provides a comprehensive assessment of Total Dissolved Solids in wastewater both before and after bacterial treatment, contributing valuable insights to the scientific understanding of water quality.

Electricity Generation

The method employed for the generation of electricity in this study involved the collection of wastewater, followed by gentle mixing to extract the maximum water with residue. Glucose, along with untreated wastewater, served as substrates to investigate the correlation between glucose consumption and the number of bacteria. A three-day experiment was conducted, assessing the impact of glucose on bacterial quantity, growth, and electricity generation. Microbial Fuel Cells (MFCs), both single and double chambers, were employed at varying temperatures (28, 30, 37, and 45 degrees Celsius) to gauge their influence on electricity production. Over a six-day period, double chambers were specifically used to evaluate the microbe's electricity-generating capabilities. Electricity generation, measured in millivolts (mV), on days 1, 2, 3, 5, and 6. The experimental setup also included subculturing eight bacteria in nutrient broth for single-MFCs, with subsequent inoculation into wastewater. To obtain generation averages, three single-MFCs were used for each bacterium, and three were dedicated to the wastewater control group. This comprehensive approach aimed to explore the potential of Microbial Fuel Cells in generating electricity from wastewater while considering different parameters such as substrate variation and temperature effects.

Procedure for assembling a single-chamber MFC:

In the construction of single-chamber Microbial Fuel Cells (MFCs), a plastic container was punctured at the bottom and another opening was made near the top, allowing for the insertion of copper wires that were subsequently sealed with a specialized adhesive. Two additional holes were created in the container lid. The compartments were then assembled, and a stainless-steel mesh, folded four times to dimensions of 10.8 cm length, 7.8 cm width, and 3 mm thickness, was constructed, and their edges were securely pressed together to prevent disassembly. Carbon batteries served also as electrodes. Copper wires were connected to the electrodes by piercing the plastic container at the top and bottom. During assembly, the anode was positioned at the container's bottom, and the cathode at the top, ensuring that one edge of each electrode made contact with wastewater, while the other faced the air. The final step involved adding wastewater to the container before sealing it, with careful attention to confirming the effective separation of the cathode and anode.

Results and Discussion

Isolation of Bacteria

The isolation of bacteria was successfully carried out, resulting in eight distinct isolates. Among these, four were identified as Gram-positive bacteria, namely *Bacillus* sp, *Actinomyces* sp, *Staphylococcus* sp, and *Micrococcus* sp, while the remaining four were classified as Gram-negative bacteria, specifically *Citrobacter* sp, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Enterobacter sakazakii*. The confirmation of isolates was achieved through biochemical testing and the utilization of specialized media. The growth patterns of these isolates on various special media were visually represented in Figures 1 and 2.

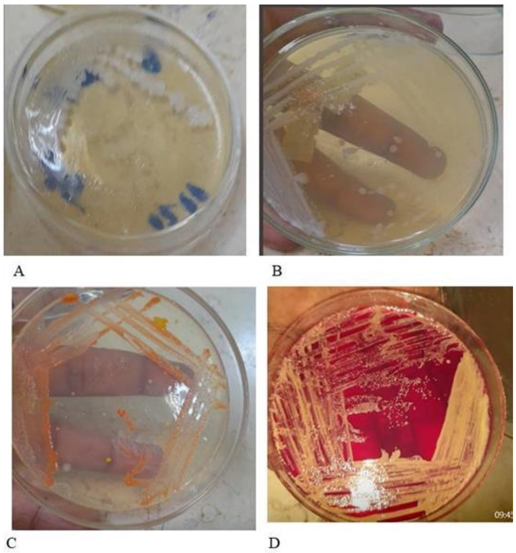

Figure 1 Development of bacterial colonies on various agar media. (A) *Bacillus* sp displaying irregular white colonies, (B) *Actinomyces* sp exhibiting large white colonies with rough features, (C) *Micrococcus* sp showcasing small orange smooth colonies on Nutrient Agar, and (D) *Staphylococcus* sp demonstrating piggie colonies on Mannitol Salt Agar.

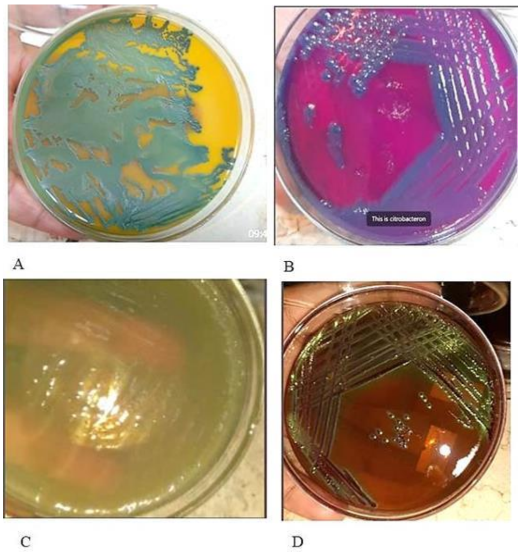

Figure 2 Growth patterns of various bacteria on specific agar media. (A) *E. aerogenes* forming emerald colonies with a color shift of the HESA media from grape to yellow. (B) *E. sakazkii* exhibiting the development of purple colonies on HESA media. (C) *P. aeruginosa* showcasing a pale green color attributed to the pyocyanin pigment on Cetrimide agar. (D) *Citrobacter* sp displaying green sheen colonies with a black center on EMB agar.

Total Suspended Solids

The modified method for quantifying total suspended solids (TSS) in wastewater, utilizing Whatman filter paper 0.45, demonstrated precision and efficiency [17]. In the assessment of untreated wastewater, the filter paper was initially weighed at 1g (W1). After filtering 100 milliliters of wastewater and drying, the re-weighing yielded a second weight of 1.4g (W2). The same process was applied to wastewater treated with bacterial growth, where filter paper weights were recorded as 1g (W1) post-treatment, and the subsequent filtered wastewater weighed 1.0244g (W2). This approach ensures accurate TSS determination, providing a comprehensive analysis of both untreated and treated wastewater samples.

Electricity Generation

In the six-day experiment using double chambers, electricity generation values were recorded as follows: 400 mV (day 1), 505 mV (day 2), 511 mV (day 3), 470 mV (day 4),), 340 mV(day 5) and 411 mV (day 6). For single-MFCs, eight bacteria were subculture in nutrient broth and added to 400 milliliters of wastewater. Three single-MFCs were used for each bacterium, and three for the wastewater control. This approach provided insights into microbial electricity generation under different conditions.

Bacterial Growth and MFC Operation Across Temperatures

In our experimental exploration of bacterial growth at different temperatures and the operation of Microbial Fuel Cells (MFCs), we confirmed the feasibility of electricity generation. Utilizing a stainless-steel single-chamber MFC for commercial use, we observed growth of isolates at 30°C, with optimal electricity generation at 37°C (1200 mV). Various anode-cathode compositions and two salt bridges were tested, revealing that a salt bridge with agar-agar in wastewater produced the best results (400 mV to 411 mV over six days) [18]. Isolated bacteria demonstrated varying electricity generation, with *Actinomyces* sp peaking at 353 mV on day 5, and *Micrococcus* sp reaching 345 mV on day 10. Mixed bacterial cultures (*Actinomyces* sp + *Bacillus* sp) achieved the highest mV on day 3 (375 mV). The electrogenic potential of *Bacillus* sp and *Actinomyces* sp, adaptable to diverse pH and temperature levels, was evident. Although most bacteria thrived at 37°C, we cultivated them at room temperature to minimize costs.

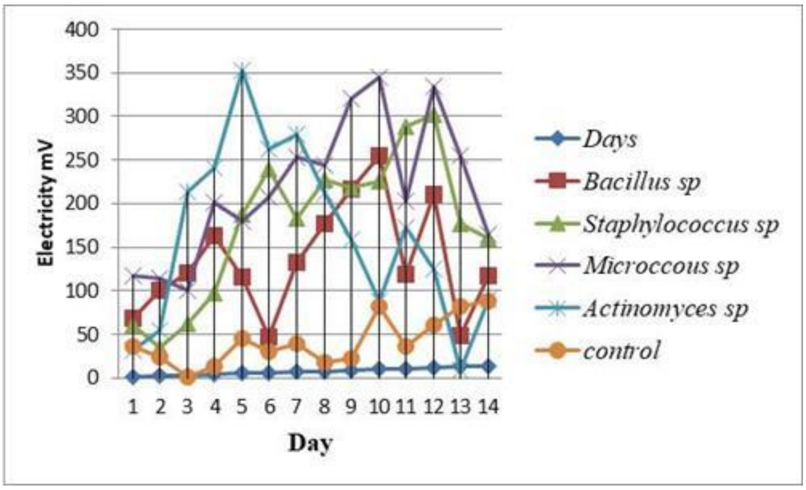

Figure 3 Electricity generation by Gram-positive bacteria, derived from the averages of three Microbial Fuel Cells (MFCs), with the addition of a glucose substrate (0.01% final concentration) on day 10 at 30°C.

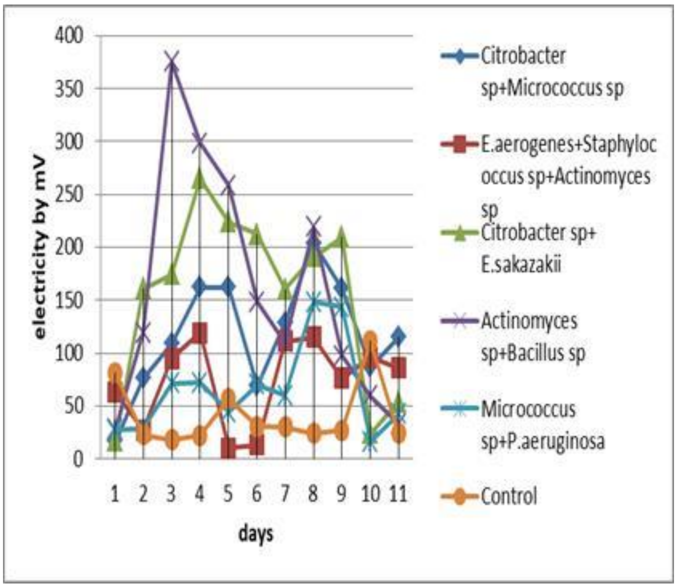

Figure 4 Electricity generation at room temperature by mixed bacterial cultures, calculated from the averages of three Microbial Fuel Cells (MFCs).

Enhancing the conductivity of cells was achieved by introducing glucose into wastewater sludge. Following this modification, bacterial isolates within individual fuel cells were allowed to respire in Microbial Fuel Cells (MFCs) for a 14-day period. Notably, on day 10, a significant increase in power generation was observed temporarily after the glucose addition. Citrobacter sp exhibited the highest mV (330 mV) (Figure 5), suggesting superior electrogenic properties compared to other bacteria. The variation in yield is attributed to fermentation, a process occurring in the presence of diverse microorganisms leading to electron loss [19].

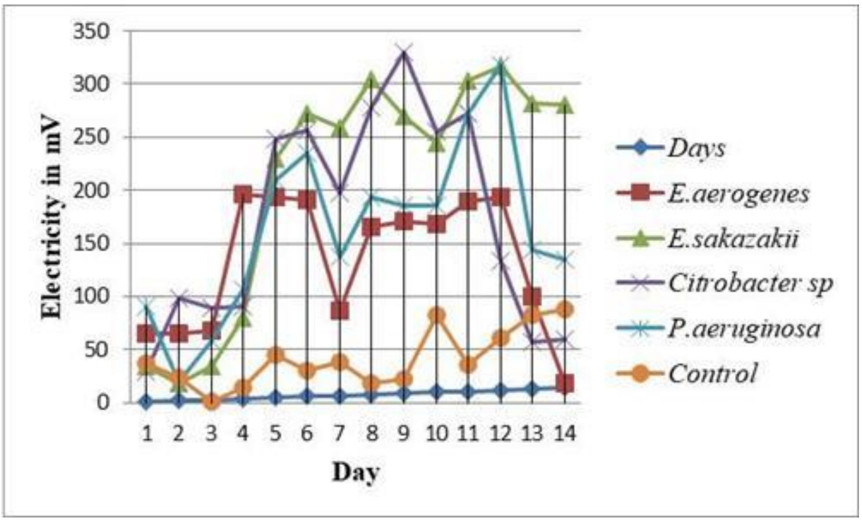

Figure 5 Average electricity generation by Gram-negative bacteria in three Microbial Fuel Cells (MFCs) following the addition of a glucose substrate (0.03%) on day 10 at 30°C.

Effect of Carbon Electrodes on Temperature-Dependent Power Generation in Microbial Fuel Cells

Results: Single-chamber Microbial Fuel Cells (MFCs) utilizing carbon battery electrodes were employed to investigate the impact of carbon electrodes on power generation at different temperatures. The experiment focused solely on wastewater, the isolates' source. Three identical wastewater samples were introduced into carbon battery single-chamber MFCs at temperatures of 45°C, 37°C, and 28°C. The highest recorded voltage, 1200mV on day 6, occurred at the optimal development temperature of 37°C, demonstrating the electrogenic bacteria's enhanced electricity generation capabilities at this temperature. Notably, carbon electrodes proved advantageous, contributing to increased generation without rust-related concerns, thus ensuring safety compared to other electrode materials. Figure 7 illustrates the detailed outcomes of the experiment.

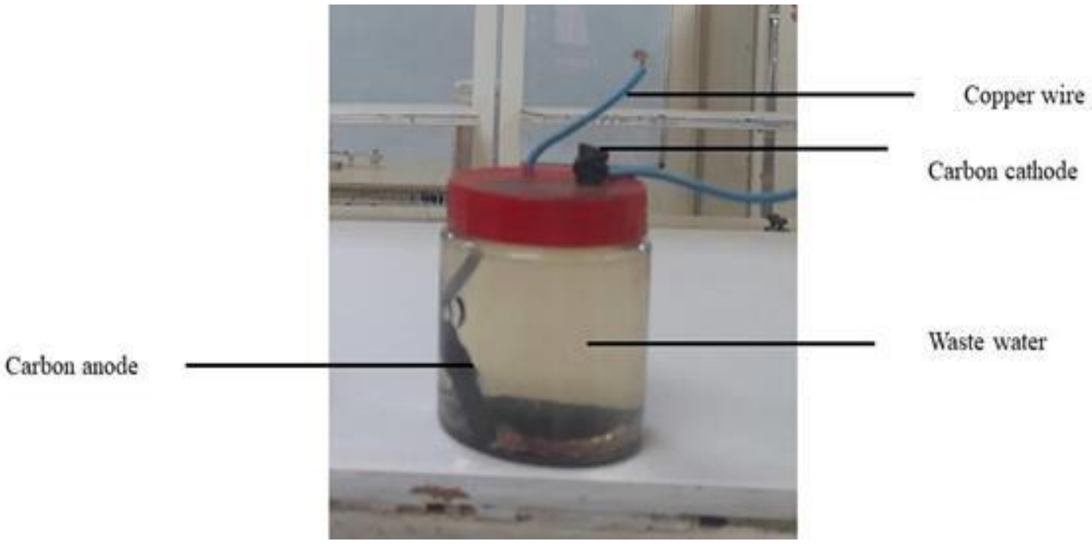

Figure 6 Single chamber MFC with carbon cathode and anode.

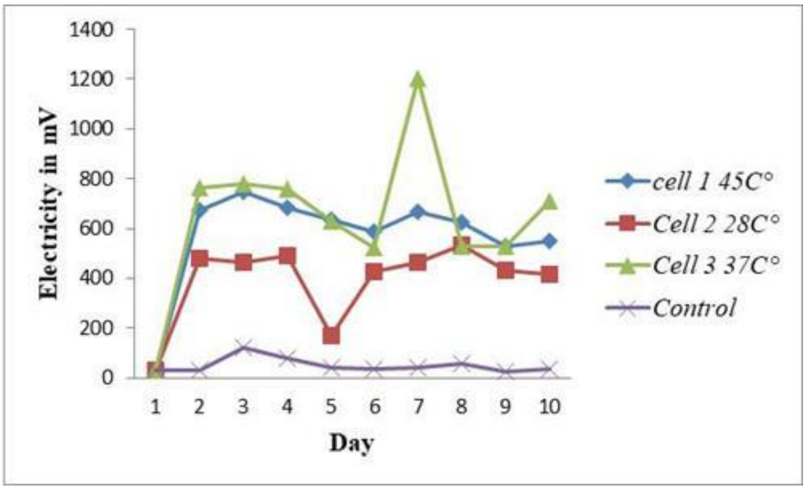

Figure 7 Electricity generation by mixed bacteria in a carbon battery electrode at varying temperatures of 45°C, 28°C, and 37°C.

Effect of Glucose Substrate on Bacterial Growth and Electricity Generation

The results from the glucose concentration experiment, as presented in the Table 1, reveal notable variations over the three-day period. The glucose standard concentrations on Day 0, Day 1, Day 2, and Day 3 were 29, 120, 80, and 44, respectively. Correspondingly, the mixed bacterial culture's voltage output (mV) demonstrated fluctuations, measuring 422, 398, 165, and 230 on the respective days. Concurrently, bacterial numbers, quantified in colony-forming units per 0.1ml (cfu/0.1ml), displayed dynamic changes at 42×10^{-4},24×10^{-4},83×10^{-4}, and $614\text{x}10^{-4}$ on Day 0, Day 1, Day 2, and Day 3, respectively. These results highlight the influence of glucose concentration on both electrical output and bacterial growth over the experimental timeframe.

Table 1 Impact of Glucose Substrate on Bacterial Growth and Electricity Generation (mV).

Glucose Concentration vs Days	Day 0	Day 1	Day 2	Day 3
Glucose Standard	29	120	80	44
Mixed Bacterial Culture (mV)	422	398	165	230
Bacterial Number (cfu/0.1ml)	42 x 10^(-4)	24 x 10^(-4)	83 x 10^(-4)	614 x 10^(-4)

Total Dissolved Solids (TDS)

The lid of a 20 ml petri plate initially weighed 45 g. After introducing 20 ml of wastewater, drying, and overnight standing [20], the re-weighed petri plate recorded a weight of 45.2 g. This process was repeated for wastewater treated with bacterial growth, maintaining the initial petri plate weight at 45 g. The measured weight of the treated wastewater was 45.0516 g using a precision balance. These findings provide insight into Total Dissolved Solids in wastewater before and after bacterial treatment.

Conclusion

In conclusion, this research establishes that electricity generation from wastewater is achievable through the isolation of bacteria without the requirement for extensive materials and high costs. The process is executed in a simple and cost-effective manner, addressing the issue of wastewater accumulation and associated problems. The quantities of electricity produced, relative to the experiment's water volume, were sufficient to power a small lamp continuously for 24 hours. This outcome underscores the efficacy of bacteria in electricity production, suggesting its potential applicability for commercial purposes.

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