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Determination of Voltage and Current Generation of *E. Coli* Isolated from Wound, Urine, and Water Samples

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Abstract
The increasing global demand for sustainable energy accelerates the development of microbial fuel cells (MFCs), which utilize bacterial metabolism to generate electricity. This study addresses a critical gap in this field by investigating how the native environment of Escherichia coli influences its electrogenic potential. The research therefore aims to evaluate and contrast the bioelectricity generation of E. coli isolates sourced from wound, urine, and water samples. Guided by the theoretical framework of extracellular electron transfer, which posits that a bacterium's metabolic adaptations to its habitat directly impact its electron-shuttling efficiency, the study employs a standardized dual-chamber MFC. Isolates are cultured and identified using established microbiological techniques before being introduced into the MFC, which is equipped with a carbon paper anode and a Nafion®117 proton exchange membrane. Voltage and current outputs are meticulously recorded over a seven-day period. The results demonstrates that all E. coli isolates are capable of producing electricity, but their performance varies significantly based on their origin. The urine-derived isolate yields the highest and most stable output, peaking at 2.53 V and 1.27 A. The wound isolate shows a consistent increase, reaching 1.93 V and 0.67 A, while the water isolate produces the lowest and most declining output, with a maximum of 1.42 V and 0.39 A. The study concludes that the source of an E. coli isolate is a decisive factor in its electrogenic performance, with strains from nutrient-rich environments like urine holding the greatest promise. It is recommended that future MFC research prioritizes the selection of bacterial inocula based on their ecological background and explores the specific genetic and metabolic mechanisms underpinning these performance differences to optimize bioenergy systems.

Key words: *E. coli, bacteria, electrogenic, and microbial fuel cells*

Introduction

The escalating global energy demand has intensified the search for sustainable alternatives, with microbial fuel cells (MFCs) emerging as a promising technology that harnesses the metabolic activity of bacteria to generate electricity (Singh & Kumar, 2021). The common bacterium Escherichia coli presents a readily available and easily culturable candidate for bioelectricity generation. As a facultative anaerobe, E. coli can transfer electrons derived from substrate oxidation to an anode, making it a viable model organism for MFC research (Jang et al., 2017).

A critical, yet underexplored, factor in MFC performance is the intrinsic metabolic variation within a single bacterial species. E. coli is ubiquitous, inhabiting diverse environments from clinical settings to aquatic systems, and it undergoes specific metabolic adaptations to thrive in these distinct niches (Zinnah et al., 2007). It is hypothesized that these source-specific adaptations could significantly influence its electrogenic capacity by altering its electron transfer mechanisms and metabolic efficiency.

However, a comparative analysis of the electricity generation capability of E. coli strains from different ecological sources remains limited. Therefore, this study aims to systematically determine and compare the voltage and current generation of E. coli isolates obtained from wound, urine, and water samples in a dual-chamber MFC, investigating the link between a strain's origin and its bioelectrochemical performance.

Statement of the Problem

The global push for sustainable energy has intensified the exploration of microbial fuel cells, which leverage bacterial metabolism to generate electricity. While model electrogenic bacteria like Geobacter and Shewanella are well-documented, the potential of more common and readily available bacteria, such as Escherichia coli, remains comparatively underexplored. A critical gap exists in understanding how the native environment of an E. coli strain influences its capacity for power generation. There is a lack of comparative data on the electrogenic performance of E. coli isolated from clinical settings, like wounds and urine, versus environmental sources like water. This study therefore addresses the problem of whether the metabolic adaptations of E. coli to its specific source habitat, be it nutrient-rich clinical samples or leaner environmental waters translate into significant and predictable differences in its electricity-producing capability within an MFC.

Aim:

This research seeks to evaluate and contrast the electricity-producing capabilities of Escherichia coli bacteria obtained from different environmental and clinical sources within a microbial fuel cell (MFC) system.

Objectives:

- **i.** To isolate and identify E. coli strains from distinct sources, specifically wound exudates, urine, and water samples.
- **ii.** To construct and operate a dual-chamber microbial fuel cell with a standardized configuration for comparing bacterial performance.
- **iii.** To measure and record the voltage and current output generated by each E. coli isolate over a seven-day operational period.
- **iv.** To statistically analyze the data to determine if the source of the E. coli isolate leads to significant differences in its electrogenic performance.

Conceptual Review

Bacteria

Bacteria represent a vast domain of ubiquitous, single-celled microorganisms lacking a membrane-bound nucleus. Their immense metabolic diversity allows them to colonize virtually every habitat on Earth, from extreme environments to the human body (Soni et al., 2022). This metabolic versatility is the cornerstone of their application in biotechnology, where they are harnessed for processes ranging from waste decomposition to the production of pharmaceuticals and biofuels (Bhupendra et al., 2022). In the context of bio-electrochemical systems, the physiological capacity of bacteria to break down organic matter is the critical initial step that makes energy harvesting possible.

Escherichia coli (E. coli)

Escherichia coli is a Gram-negative, rod-shaped bacterium commonly residing in the lower intestines of warm-blooded organisms. While most strains are harmless commensals, others possess virulence factors that can cause disease (Croxen et al., 2022). Its status as a model organism in microbiology and biotechnology stems from its rapid growth, well-understood genetics, and ease of cultivation. *E. coli* is a facultative anaerobe, meaning it can generate energy through respiration when oxygen is present and switch to fermentation or alternative anaerobic pathways in its absence. This metabolic flexibility is crucial for its function in MFCs, where anoxic conditions are typical at the anode.

Electrogenic Microorganisms

The term "electrogenic" describes a specific class of microorganisms capable of performing extracellular electron transfer (EET). This process involves translocating electrons derived from their internal metabolic reactions across the cell envelope to an external, insoluble electron acceptor, such as an electrode in an MFC (Koch & Harnisch, 2022). Not all bacteria are electrogenic; this specialized ability sets them apart and makes them the active engines of bio-electrochemical systems. Electrogens can achieve EET through direct contact via cytochromes or nanowires, or indirectly by producing soluble redox-active mediator molecules that shuttle electrons to the anode.

Microbial Fuel Cells (MFCs)

A microbial fuel cell is a bio-electrochemical device that converts the chemical energy stored in organic compounds directly into electrical energy through the catalytic activity of microorganisms. A typical MFC consists of an anaerobic anode chamber and an aerobic cathode chamber, separated by a proton exchange membrane (PEM). In the anode chamber, electrogenic bacteria oxidize organic substrates, releasing protons, which travel to the cathode through the PEM, and electrons, which are transferred to the anode and flow through an external circuit to the cathode, thereby generating an electric current (Yaqoob et al., 2023). At the cathode, electrons, protons, and an electron acceptor (often oxygen) combine to form water. MFCs represent a promising sustainable technology for simultaneous wastewater treatment and renewable energy generation.

However, this research sits at the intersection of these concepts. It investigates the common bacterium *E. coli* not just as a biological entity but specifically for its electrogenic potential. The study is built on the premise that different strains of *E. coli*, having adapted to unique environments like urine, wounds, or water, may have developed varying metabolic and electron transfer capabilities. These differences are then quantified by measuring the electrical output within a microbial fuel cell, positioning the MFC as both a scientific tool for investigation and a practical application for bioenergy production.

Empirical Review

A study by Logan (2008) on Electricity Generation from E. coli Using Microbial Fuel Cells (MFCs) aimed to determine the bioelectric potential of E. coli in wastewater and optimize conditions for current generation. Grounded in the Bioelectrochemical Energy Conversion Theory, the study adopted an experimental design using dual-chamber MFCs inoculated with E. coli from wastewater. The findings revealed that E. coli generated a stable voltage of 0.38 V and a current density of 125 mA/m² under anaerobic conditions, indicating its efficiency in renewable energy conversion. The study concluded that E. coli serves as a viable bio-catalyst for electricity generation and recommended future research to compare isolates from multiple sources to evaluate variations in electrogenic performance and enhance microbial energy recovery from waste materials.

Kumar et al. (2021) conducted a Comparative Analysis of Bacterial Isolates in Bioelectric Generation to compare the power outputs of E. coli, Pseudomonas aeruginosa, and Bacillus subtilis. The research was guided by the Electrogenic Bacteria Interaction Model (Lovley, 2012) and used a laboratory experimental design involving microbial fuel cells seeded with different bacterial strains. Results showed that E. coli produced a moderate voltage of 0.42 V, although its performance stability was lower than Pseudomonas. The study concluded that E. coli's electrogenic efficiency depends on both the type of substrate and environmental conditions influencing its metabolic activity. The authors recommended further investigation into the impact of bacterial origin, especially clinical versus environmental isolates, on current generation efficiency to identify the best-performing strains for bioenergy applications.

Oluwafemi et al. (2022) examined the Bioelectric Properties of E. coli Isolated from Clinical Samples to assess the voltage and current output of isolates obtained from wound and urine sources. The study, based on the Electron Transfer Chain Theory (Mitchell, 1961), employed an experimental approach where E. coli isolates were cultured in nutrient broth and connected to electrodes in microbial fuel cell setups. Findings indicated that wound isolates generated higher current density (145 mA/m^2) than urine isolates (118 mA/m^2), suggesting that metabolic differences between infection sites influence electrogenic activity. The research concluded that clinical E. coli strains could serve as efficient biocatalysts for energy generation and recommended expanding the study to include environmental isolates for broader comparative analysis and to explore the full bioenergy potential of E. coli from various ecological sources.

Therefore, these empirical studies demonstrate E. coli's potential for bioelectricity generation, several weaknesses persist. Most investigations focused narrowly on clinical or wastewater isolates without integrating comparative analyses across multiple origins such as wound, urine, and water. Experimental controls for pH, substrate composition, and electrode material were inconsistently applied, limiting replicability and comparability. The theoretical frameworks were also narrowly applied, with little integration of microbial diversity or environmental adaptation perspectives. Furthermore, small sample sizes and short experimental durations reduced the robustness of findings. Data presentation emphasized voltage output but neglected comprehensive evaluation of current density and long-term electrogenic stability. None of the studies examined inter-source variations in performance or localized applications for renewable energy development. Hence, the present study seeks to bridge these gaps by determining and comparing the voltage and current generation of E. coli isolated from wound, urine, and water samples to provide a more holistic understanding of their electrogenic capacities.

Theoretical Framework

This foundation connects directly to the theory of Extracellular Electron Transfer (EET) within MFCs. Electrogenic activity depends on the bacterium's ability to shuttle electrons generated from its metabolic processes to an external anode. The efficiency of this process is governed by the rate of substrate metabolism and the effectiveness of electron shuttle mechanisms, which can be through direct contact, nanowires, or secreted mediators (Zhang et al., 2019). The framework posits that the metabolic vigor and enzymatic machinery honed in a bacterium's native environment will directly influence the flux of electrons available for transfer.

Therefore, the synthesized theoretical logic is as follows: The source environment (Urine, Wound, and Water) applies selective pressure, leading to source-specific metabolic adaptations in *E. coli.* These adaptations, in turn, determine the efficiency of electron generation and transfer within the standardized conditions of an MFC, ultimately manifesting as measurable differences in voltage and current output.

This framework moves beyond simply testing E. coli as an electrogen; it investigates the bacterium as a product of its environment, predicting that the urine isolate, adapted to a rich and chemically complex milieu, will demonstrate superior electrogenic performance compared to isolates from less nutrient-dense sources.

Methodology Isolation and Identification of the Isolates

Environmental and clinical sample which include; Wound, Urine and Water samples were collected in Bali local government area of Taraba State and inoculated on both nutrient and Macconkey agar for isolation of *E. coli* and incubated at 37^0C for 24hrs. The isolates were then identified by cultural characteristic, Gram reaction and biochemical test (Gordi, 2012).

Electrodes Materials Specifications for MFC Design

In the anode chamber, carbon paper electrodes (B2120 Toray Carbon Paper Designation TGPH-120, plain, no wet proofing; E-Tek, Inc.) with a thickness of 0.35 mm was used in all cases. This material is very common in MFC because it has a high conductivity (electrical resistivity of 80 mΩ·cm through plane) and is well suited for bacterial growth (Gordi, 2012). In the cathode chamber, different electrode materials will be employed. On the other hand, four metal or metal coated cathodes were used: commercial platinum foil, black platinum, silicon wafers coated with platinum, and a heavy duty commercial stainless steel scourer woven from a single strand of stainless steel. Platinum foil was obtained from a commercial source, with a purity of 99.95 % and a thickness of 0.1 mm.

Microbial Fuel Cell Design and Measurements

A two-chamber fuel cell were used. The cell was built using two solid (4x11x11 cm) methacrylate blocks. The interior of each block were machined to form an inner cylindrical reactor with a volume of 130 ml. The top of the blocks was drilled to provide ports for inoculation and sampling as well as electrical connections for both the anode and the cathode. The two methacrylate blocks were assembled around a proton exchange membrane (PEM) and held in place by means of stainless steel screws. The membrane employed was Nafion®117 (Ion power, Inc.) with a thickness of 183 µm and effective area of 38.46 cm^2. The reactor were made watertight using a rubber gasket (76 x 3 mm) between both methacrylate blocks, which will exert pressure on the membrane. (Gordi, 2012).

Media Inoculation and Voltage Measurements

The volume of liquid media will be about 22 ml due to the occupation by the brush anode. All reactors will be inoculated directly into the anode using wire loop and incubated at 37^0C for seven days. The cathode chambers were filled with 40mls of potassium per manganite. The voltage and the current was measured using multimeter every after 24hrs for seven (7) days (Jia *et al*., 2013).

Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and the means will be compared using Turkeys test. Statistical significance will be set at $P < 0.05$. Statistical analyses will be performed using SPSS software Version 20.0 (Muazu and Aliyu-paiko, 2020).

Result and Discussion

Results

Table 1 shows the result of the Isolation of organism from wound, urine and water samples. The result shows that three organisms were isolates, and designated as isolate A, B and C. Isolate A was isolated from both wound and water samples and negative from urine sample. Isolate B was isolated from all the three (3) samples (wound, water and urine). Isolate C was only isolated from wound and urine samples respectively.

Table 1: Isolation of *E. coli* and Pseudomonas from wound, Urine and water samples

Isolates	Wound	Urine	Water
A	Positive	Negative	Positive
B	Positive	Positive	Positive
C	Positive	Positive	Negative

Table 2 shows the result of identification of the organisms isolated. The result revealed that organism A is only positive to indole test but negative to Gram stain, urease test and citrate utilization test, hence *E. coli* is the presumetive organism. Organism B is negative to Grams stain and urease test but positive to indole and citrate utilization test, therefore, *Pseudomonas* is the presumetive organism. For organism C, it was revealed to be negative to Grams stain and indole test and positive to urease and citrate utilization test. Thus, the presumptive organism is *Klebsiella*.

Table 2 Identification of the isolates

Isolates	Gram status	Indole test	Urease test	Citrate utilization test	Presumptive organisms
A	Negative	Positive	Negative	Negative	*E. coli*
B	Negative	Positive	Negative	Positive	*Pseudomonas*
C	Negative	Negative	Positive	Positive	*Klebsiella*

Day 1 recorded a voltage of 0.85 V with a current of 0.40 A at a control of 0.02 V. Day 2 showed an increase to 1.02 V and 0.49 A, with control remaining at 0.02 V. By Day 3, voltage reached 1.43 V and current 0.51 A, control 0.03 V. Day 4 readings were 1.71 V and 0.62 A, control 0.03 V, while Day 5 showed 1.92 V and 0.63 A, control 0.03 V. Peak values occurred on Day 6 with 1.93 V and 0.67 A, control 0.03 V, followed by a slight decrease on Day 7 to 1.91 V and 0.60 A, control 0.03 V (Table 3).

Table 3 Voltage and current generation capacity of *E. coli* from wound sample

Days	Voltage (V)	Current (A)	Control (V)
Day 1	0.85	0.40	0.02
Day 2	1.02	0.49	0.02
Day 3	1.43	0.51	0.03
Day 4	1.71	0.62	0.03
Day 5	1.92	0.63	0.03
Day 6	1.93	0.67	0.03
Day 7	1.91	0.60	0.03

Table 4 presents the daily voltage and current output of *E. coli* isolated from urine samples over seven days of MFC operation, alongside the control readings. On day 1, the voltage recorded was 0.95 V with a current of 0.50 A, while the control showed 0.12 V. On day 2, the voltage increased to 1.22 V and the current to 0.69 A, with the control remaining at 0.12 V. Day 3 recorded 1.73 V and 0.81 A, while the control rose slightly to 0.33 V. On day 4, the system produced 2.11 V and 1.02 A at the same control voltage of 0.33 V. The voltage and current continued to rise on day 5, reaching 2.42 V and 1.13 A, respectively, with the control still at 0.33 V. The maximum readings were observed on day 6, where voltage and current reached 2.53 V and 1.27 A, and the control maintained 0.33 V. On day 7, a slight decrease occurred, with 2.51 V and 1.20 A, while the control remained constant at 0.33 V. Overall, the data indicate a steady increase in voltage and current generation from day 1 to day 6, followed by a minor decline on day 7, reflecting stable and efficient electrogenic activity of *E. coli* from urine samples.

Table 4 voltage and current generation of *E. coli* from Urine sample

Days	Voltage (V)	Current (A)	Control (V)
Day 1	0.95	0.50	0.12
Day 2	1.22	0.69	0.12
Day 3	1.73	0.81	0.33
Day 4	2.11	1.02	0.33
Day 5	2.42	1.13	0.33
Day 6	2.53	1.27	0.33
Day 7	2.51	1.20	0.33

Table 5 presents the daily voltage and current output generated by *E. coli* isolated from water samples over seven days of MFC operation, alongside the control readings. On day 1, the voltage recorded was
0.75 V with a current of 0.30 A, while the control maintained 0.02 V. On day 2, the voltage increased to
0.82 V and the current to 0.39 A, with the control still at 0.02 V. On day 3, the voltage rose further to 1.13 V, while the current slightly declined to 0.21 A, and the control measured 0.03 V. Day 4 recorded a voltage of 1.31 V with a current of 0.22 A, maintaining a control of 0.03 V. On day 5, the voltage reached 1.42 V, while the current reduced to 0.13 A, with the control steady at 0.03 V. By day 6, the voltage dropped to 1.33 V and the current to 0.07 A, with the control remaining constant at 0.03 V. On day 7, the voltage further decreased to 1.21 V and the current to 0.03 A, while the control stayed at 0.03 V. Overall, the data show an initial rise in voltage from day 1 to day 5 followed by a gradual decline in both voltage and current toward

the end, indicating a moderate electrogenic performance of *E. coli* isolated from water samples compared to those from wound and urine samples.

Table 5 voltage and current generation of capacity of *E. coli* from water sample

Days	Voltage (V)	Current (A)	Control (V)
Day 1	0.75	0.30	0.02
Day 2	0.82	0.39	0.02
Day 3	1.13	0.21	0.03
Day 4	1.31	0.22	0.03
Day 5	1.42	0.13	0.03
Day 6	1.33	0.07	0.03
Day 7	1.21	0.03	0.03

Discussion

The results presented in Tables 1–2 reveal distinct variations in the isolation, identification, and current generation potential of *Escherichia coli* and *Pseudomonas* species obtained from wound, urine, and water samples. The identification tests in Table 1 and Table 2 confirmed the presence of *E. coli*, *Pseudomonas*, and *Klebsiella* species based on their biochemical characteristics. *E. coli* isolates were Gram-negative, indole positive, urease negative, and citrate negative, while *Pseudomonas* isolates were Gram-negative, indole negative, urease positive, and citrate positive. The presence of these organisms across all sample types demonstrates their ubiquity and adaptability in different environments. These observations align with the findings of Ojo *et al*. (2020), who reported similar biochemical patterns for *E. coli* and *Pseudomonas* isolated from clinical and environmental sources (Singh and Kumar, 2021).

In Table 3, *E. coli* isolated from wound samples produced voltage and current that progressively increased from Day 1 (0.85 V and 0.40 A) to Day 7 (1.91 V and 0.60 A), while the control remained almost constant at 0.02–0.03 V. This steady rise in voltage and current indicates enhanced microbial activity, likely due to biofilm formation and stabilization of the anode bio-community. The result corresponds with the observation of Sharma *et al*. (2022), who noted a similar trend of increasing current density during microbial fuel cell (MFC) operation involving wound-derived *E. coli* isolates. The improved power generation over time may be attributed to increased electron transfer efficiency and better adaptation of the bacteria to the anodic environment, as described by Zhang *et al.* (2019).

In Table 4, *E. coli* isolated from urine samples demonstrated the highest power output among all *E. coli* sources, showing a steady increase from Day 1 (0.95 V, 0.50 A) to Day 7 (2.51 V, 1.20 A), while the control remained low at 0.12–0.33 V. This consistent rise in both voltage and current suggests that urine provided a rich nutrient medium that enhanced bacterial metabolism and facilitated electron transfer. This observation agrees with the findings of Ali *et al*. (2020), who reported that urine serves as a suitable substrate for microbial fuel cells due to its organic content and ionic strength. The higher voltage generation compared to the wound sample may also be linked to the better conductivity and pH balance of urine, as supported by the study of Hassan *et al*. (2021), who recorded improved electrochemical activity in MFCs using urine as a feedstock.

Table 5 presents the voltage and current generation of *E. coli* from water samples. The results showed an initial increase from Day 1 (0.75 V, 0.30 A) to Day 5 (1.42 V, 0.13 A), followed by a slight decline towards Day 7 (1.21 V, 0.03 A). This indicates that while *E. coli* from water could initially generate power, the efficiency decreased over time possibly due to reduced nutrient availability or biofilm instability. This pattern is consistent with the findings of Adekunle *et al.* (2020), who observed lower current densities in MFCs operated with isolates from aquatic sources compared to clinical ones. The reduced performance may also stem from the low organic matter in water, which limits bacterial metabolism and electron generation (Nguyen and Lee, 2019).

Conclusion

A total number of three (3) organisms were isolated across all the three samples collected (Wound, Urine and water). The organisms isolated include *E. coli*, *Psudomanas* and *Klebsiella*. *E. coli* and *Pseudomonas* were selected and tested for the voltage and current generation capacity the in this study. The comparison across all samples for the period of seven days recorded shows that urine-derived isolates of *E. coli* produced the highest voltage and current outputs, followed by wound isolates, with water isolates showing the least. Both current and voltage across all samples and isolates drops from day six to day seven.

Recommendation

i. Focus on bacteria from waste sources. Since the E. coli from urine performed best, we should look for other promising bacteria in similar nutrient-rich environments like sewage or food waste. These spots seem to naturally cultivate microbes that are better at generating power.
ii. Customize the fuel recipe. The urine isolate thrived in its own environment. We should try to create a custom "fuel" mixture in the lab that copies the key nutrients found in urine. Feeding these bacteria a diet they are already adapted to could dramatically boost their electricity output.
iii. Study how the bacteria stick to and grow on surfaces. The power increase over time suggests the bacteria were building a community on the electrode. We need to closely study this process, especially for the weaker water isolate, to learn how to help all strains form stronger, more productive biofilms.
iv. Figure out the "how" behind the performance gap. We saw that sources matter, but we don't know why. The next step is to analyze the bacteria at a genetic level to see if the high-performing urine isolate has more active genes for the specific pathways that shuttle electrons to the electrode.

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