

# Synergistic Valorization: Generating Bioelectricity and High- Protein Animal Feed From Fermented Crop Residues

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## Research Article

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## Abstract

## Background

The microbial communities present in agricultural wastes and their potential for bioelectricity generation through microbial metabolism warrant exploration for potential synergistic biotechnological applications.

## Methods

Utilizing a combination of culture, biochemical assays, and 16S rRNA sequencing, known and novel microorganisms within maize husk, sweet potato peel, wheat shaft, and sugar cane shaft substrates were identified. The proximate, mineral, and vitamin content of the agro-wastes were determined before and after fermentation to ascertain the agro-waste substrate suitability for animal feed. A dual-chamber microbial fuel cell (MFC) was constructed to evaluate the bioelectricity generation potential of these substrates over 21 days.

## Results

Potato peel exhibited the highest bacterial count at  $3.8 \times 10^4$  CFU/ml, while maize husk had the highest fungal load at  $6.55 \times 10^4$  SFU/ml. Fermentation significantly enhanced the protein, mineral, and vitamin content of the agrowastes while reducing fiber and carbohydrate levels. Notably, maize husk produced the highest voltage of 68 mV and current of 77  $\mu$ A compared to other substrates. The mixed culture of *L. fusiformis* and *B. subtilis* also demonstrated substantial voltage and current outputs from maize substrate during days 1–6. *Pichia kudriavzevii* MN007220.1, *Geotrichum candidum* MK943778.1, *Bacillus subtilis* NR102783.2, and *Lysinibacillus fusiformis* KP419973.1 were confirmed in these agro-waste substrates.

## Conclusions

These findings underscore the dual potential of agricultural wastes as a valuable source for bioelectricity generation and as a nutritious supplement for animal feed post-fermentation, aiding digestibility. This emphasizes their importance in sustainable agricultural practices and biotechnological applications.

## Background

The escalating global population and the intensification of agricultural activities have led to the generation of vast quantities of agro-industrial wastes, posing significant environmental and logistical challenges (Saini et al., 2021). The improper disposal of these residues, such as through open burning or landfilling, contributes to greenhouse gas emissions and resource depletion, running counter to global sustainability objectives (Saini et al., 2021; Sileshi et al., 2025). In response, the concept of a circular bioeconomy has gained prominence, advocating for a paradigm shift from a linear "take-make-dispose" model to a regenerative one (Okolie et al., 2023; Pinar et al., 2025). This approach champions "Waste-to-Wealth" strategies, wherein agricultural by-products are repurposed as valuable feedstocks for producing bioenergy, biochemicals, and nutritionally enhanced animal feed (Sileshi et al., 2025; Pinar et al., 2025). Such valorization pathways not only mitigate the environmental burden of agricultural production but also enhance food security and create new economic opportunities, directly aligning with Sustainable Development Goals (SDGs) for affordable and clean energy (SDG 7) and sustainable communities (SDG 11) (Banu et al., 2023; Al-Badani et al., 2024).

Among the diverse valorization technologies, microbial fermentation and bio-electrochemical systems represent two powerful and potentially synergistic platforms. Solid-state fermentation (SSF) has emerged as a highly effective and environmentally benign method for upgrading the nutritional value of lignocellulosic agro-residues for use in animal feed (Carranza-Méndez et al., 2025; Eze et al., 2025). These residues are often characterized by low protein content, high fiber, and the presence of anti-nutritional factors, which limit their direct use (Carranza-Méndez et al., 2025). Recent advancements have demonstrated that SSF, utilizing robust fungal and bacterial strains, can significantly increase crude protein content through the proliferation of microbial biomass (single-cell protein), enhance digestibility by enzymatically breaking down complex fibers like cellulose and hemicellulose, and detoxify the substrate by degrading anti-nutritional compounds (Carranza-Méndez et al., 2025; Eze et al., 2025; Ji et al., 2025). This bioprocessing step transforms low-value by-products into high-quality, protein-rich feed ingredients, reducing reliance on conventional and often costly protein sources like soybean meal (Eze et al., 2025).

Concurrently, microbial fuel cells (MFCs) have garnered significant attention as a promising technology for generating bioelectricity directly from the chemical energy stored in organic waste (Hossain et al., 2022; Banu et al., 2023). An MFC is a bio-electrochemical device that utilizes electroactive microorganisms to oxidize organic substrates, producing electrons and protons that generate an electrical current (Hossain et al., 2022; Al-Badani et al., 2024). This process offers the dual benefit of waste treatment and sustainable energy production from a carbon-neutral source (Hossain et al., 2022). Despite their potential, the widespread application of MFCs faces challenges, primarily related to low power output and the efficiency of microbial catalysis (Hossain et al., 2022; Al-Badani et al., 2024). Consequently, research efforts are increasingly focused on optimizing system components, particularly the anode electrode and the composition of the electrogenic microbial biofilm that colonizes its surface, as this is the primary site of biological oxidation and electron transfer (Hossain et al., 2022; Dessie and Tadesse, 2022).

The efficacy of both SSF and MFCs is fundamentally dependent on the metabolic activities of the resident microbial communities. In MFCs, a specialized group of microorganisms known as exo-electrogens or electrochemically active bacteria (EAB) are the primary drivers of power generation (Al-Badani et al., 2024). These bacteria possess unique extracellular electron transfer (EET) mechanisms that allow them to transfer electrons, generated from substrate

oxidation, to an external, insoluble electron acceptor like the MFC anode (Al-Badani et al., 2024). However, the complex polymeric structure of lignocellulosic agro-wastes, composed mainly of cellulose, hemicellulose, and lignin, presents a metabolic bottleneck. Exo-electrogens typically utilize simple sugars, organic acids, or alcohols as electron donors and cannot directly metabolize these complex polysaccharides (Guldhe et al., 2023; Yan et al., 2025).

Therefore, the efficient conversion of raw agro-wastes in an MFC necessitates a syntrophic microbial consortium with diverse and complementary metabolic capabilities (Guldhe et al., 2023). This consortium requires a hydrolytic "front-end," comprising bacteria and fungi that secrete a suite of extracellular enzymes (e.g., cellulases, hemicellulases) to deconstruct the complex biomass into fermentable monomers (Guldhe et al., 2023; Yan et al., 2025). These monomers are then fermented by other members of the community into simpler organic acids and alcohols, which subsequently serve as the primary fuel for the exo-electrogenic bacteria at the anode. This multi-step biochemical cascade underscores a critical synergy: the same hydrolytic and fermentative processes that enhance the nutritional value of agro-wastes for animal feed are precisely those required to liberate the low-molecular-weight substrates needed for efficient bioelectricity generation. The composition of the initial agro-waste, whether rich in readily available starch like potato peels or complex lignocellulose like maize husks, will thus act as a powerful selective pressure, shaping the structure of the microbial community and ultimately dictating the efficiency of both valorization pathways.

While fermentation for enhanced animal feed and MFCs for bioelectricity generation are often investigated as separate processes, there is a compelling rationale for exploring their integration within a single, synergistic system. The potential to harness a single indigenous microbial consortium to simultaneously produce two distinct value-added products from a common waste stream represents a significant advancement in circular bioeconomy principles. However, there remains a knowledge gap concerning the performance of native microbial communities from diverse agro-wastes in such dual-purpose systems. A deeper understanding of the key microbial players and their specific contributions to both substrate degradation and electron transfer is essential for process optimization. Therefore, this study was conducted to assess the dual valorization potential of four common agro-wastes: maize husk, sweet potato peel, wheat shaft, and sugarcane shaft. The specific objectives were to characterize the changes in nutritional composition of the wastes following fermentation evaluate their bioelectricity generation potential in a dual-chamber MFC; and identify the dominant microbial species responsible for these transformations, thereby elucidating their potential for synergistic biotechnological applications.

## Methods

### Collection of agro-wastes

Maize husk, sweet potato peels, wheat shaft and sugar cane shaft were all aseptically collected from different agricultural waste sites within Akure South Local government area of Ondo State, Nigeria. They were kept separately in sterile air tight polythene bags and transported to the Microbiology Laboratory, Federal University of Technology, Akure, Nigeria, for further analysis within 24 hours.

### Preparation of agro-waste substrate samples

The substrates were dried in the drying cabinet for a period of 14 days after which they were milled individually into powder using an electric blender (Binatone blender/grinder- BLG 450).

### Initial Isolation of Bacteria and Fungi from agro-waste samples

The initial isolation of bacteria and fungi from agricultural waste samples utilized a systematic serial dilution method to decrease microbial populations. One gram of each sample was diluted in 9 mL of sterile distilled water, followed by stepwise dilutions to achieve specific dilution factors ( $10^{-5}$  for bacteria and  $10^{-3}$  for fungi). A 1 mL aliquot from these dilutions was plated on nutrient media using the pour plate technique and incubated at 37 °C for 24 hours for bacteria and at 25 °C for 48 to 72 hours for fungi. After incubation, bacterial colonies were sub-cultured for pure culture isolation, while fungal colonies underwent similar procedures. The pure isolates were stored at 4 °C for future use.

### Enumeration of Bacterial Colony and Fungal Spore

Colony and spore counting were carried out visually by counting the number of visible colonies/spores that appeared on the plates; a plate that has a distinct colony/spore was used (Carranza-Méndez et al., 2025). Calculation of colony forming unit (CFU) per ml and spore forming unit (SFU) per ml for bacteria and fungi respectively was based on the formula:

$$CFU/SFU= \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Number of mL suspended}}$$

### Cultural Characterization and Identification of Bacterial Isolates

Cultural characteristics of discrete bacterial colonies, including color, shape, pigmentation, elevation, margin, texture, and opacity, were observed after 24 hours of incubation. Microscopic characterization was performed using the Gram staining procedure, while biochemical tests were conducted following established methods (Oladiipo et al., 2024).

### Morphological Characterization of Fungi and Yeasts from Agricultural Wastes

Distinct moulds isolated from agricultural wastes were purified by sub-culturing on freshly prepared potato dextrose agar plates and incubated at 25 °C for 5-7 days. The morphological and cultural characteristics of the fungal isolates were examined based on the color, types, and shapes of spores, conidia, and hyphae. The isolates were stained with lactophenol-cotton blue dye and viewed under a light microscope to assess conidia shape, sporangiophore, arthrospores, spore head, rhizoid, and hyphae (septate or non-septate) (Kamilari et al., 2023). For yeast characterization, isolated yeasts were biochemically tested for their ability to ferment sugars, utilize nitrate, and form structures such as spores, mycelium, pseudomycelium, and pellicles. Morphological characterization involved staining smears of yeast isolates with lactophenol-cotton blue dye and examining them under a light microscope using oil immersion. The Dalmat plate procedure was employed to assess pseudomycelium and mycelium production on corn-meal agar. Observations were made daily for up to five days to differentiate between filamentous hyphal growth (mycelium) and budding cells (pseudomycelium). To evaluate ascospores formation, wet mounts of yeast isolates from potato dextrose broth were stained with malachite green and safranin. This staining process allowed for the visualization of spore shapes, where spores appeared green and vegetative cells stained pink-red (Kamilari et al., 2023).

## **Fermentation of yeast isolates from agro-waste via Sugar Fermentation and Nitrate Utilization tests**

The technique outlined by Chu et al. (2023) was employed to evaluate the fermentation capabilities of various yeast isolates and their ability to utilize nitrate. For sugar fermentation, carbohydrates such as glucose, fructose, sucrose, maltose, galactose, and lactose were tested at a concentration of 10% (w/v) in a peptone mineral medium with phenol red as a pH indicator. The medium was autoclaved, cooled, and inoculated with a loopful of 24-hour-old yeast culture before incubation at 25 °C for 48 hours. Gas production was monitored using Durham tubes, while acid production was indicated by a color change from red to orange (Chu et al., 2023). For nitrate utilization, a medium containing potassium nitrate and peptone was prepared and autoclaved. Each test tube was inoculated with a 24-hour-old yeast culture and incubated at 25 °C for 72 hours. After incubation, nitrate presence was assessed by adding standard nitrate test reagents; the development of a pink color indicated nitrate reduction.

## **DNA Extraction, Amplification, and Sequencing of Bacterial and Fungal Isolates from Agricultural Wastes**

### **Bacterial Isolates**

DNA extraction from bacterial isolates was performed using the Jena Bioscience Bacteria DNA Preparation Kit (PP-206, Jena Bioscience, Jena, Germany). Bacterial isolates were first cultured in Luria Broth (LB) at 37 °C with shaking for 36 hours. One milliliter of each pure bacterial isolate was centrifuged to obtain a pellet. The pellet was resuspended, and cells were lysed, followed by protein precipitation. The supernatant containing DNA was transferred to a new tube, and DNA was precipitated by adding isopropanol. The mixture was centrifuged to form a DNA pellet, which was washed, air-dried, and resuspended in DNA Hydration Solution. The extracted DNA was stored at 4 °C (Liu et al., 2024). DNA quantification was conducted by measuring absorbance at 600 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). PCR amplification of the bacterial 16S rRNA gene was performed using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTTGTACGACTT-3'). Thermal cycling was carried out in an Applied Biosystems Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) with the following conditions: initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 1.5 minutes, and a final extension at 72 °C for 10 minutes (Liu et al., 2024). The amplified DNA fragments were visualized on a 1% agarose gel stained with ethidium bromide. Amplicons were purified using a sodium acetate wash technique. Sequencing analysis was performed by mixing the purified amplicons with HiDi formamide and loading onto an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (Oladipo et al., 2024).

### **Fungal Isolates (Yeast strains)**

DNA extraction from fungal isolates (yeast) was performed using the Jena Bioscience Animal and Fungi DNA Preparation Kit (PP-208, Jena Bioscience, Jena, Germany). Fungal isolates were cultured on YPD agar at 28 °C for 48-72 hours. The DNA extraction procedure followed the same steps as for bacterial isolates (Elhalis et al., 2021). DNA quantification was conducted as described for bacterial isolates. PCR amplification of the fungal ITS region was performed using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Thermal cycling was carried out with the following conditions: initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 1 minute, and a final extension at 72 °C for 10 minutes (Okane et al., 2025). The amplified DNA fragments were visualized on a 1% agarose gel, and amplicons were purified using the same sodium acetate wash technique as for bacterial isolates. Sequencing analysis was performed as described for bacterial isolates (Okane et al., 2025).

## **Proximate analyses of agro-waste substrates**

Moisture content was assessed using the oven drying method (Nath et al., 2024). Ash content was determined by placing a sample in a Nabertherm muffle furnace at 600 °C. The Kjeldahl method was employed to determine crude protein content through nitrogen quantification (Eze et al., 2025). Crude fat content was determined using the ether extract method via Soxhlet extraction apparatus. To determine crude fiber content, a sample was sequentially boiled in 1.25% H<sub>2</sub>SO<sub>4</sub> and 1.25% NaOH solution (Carranza-Méndez et al., 2025). Total carbohydrate content was assessed by subtracting the sum of moisture, crude fat, crude protein, and crude fiber percentages from 100 (Nath et al., 2024).

## **Determination of mineral compositions of agro-waste substrates**

Calcium content was determined using ethylene-diaminetetraacetic acid (EDTA) complexometric titration. Potassium (K) and sodium (Na) contents were analyzed using a Systronics model 130 flame photometer. Zinc (Zn), Lead (Pb), and Iron (Fe) content were assessed via atomic absorption spectrophotometry (AAS) after appropriate sample digestion (Olorunnisola and Onwudili, 2024).

## **Determination of vitamin content of samples**

The vitamin content of agricultural waste samples was determined using standardized methodologies for various vitamins, including vitamins C, B1, B2, D, and A, with absorbance measured at specific wavelengths for quantification (Eze et al., 2025).

**Determination of ethanol and organic acid contents of agro-wastes**

The ethanol content of agro-waste samples was determined by distillation and measurement of specific gravity (Okolie et al., 2023). Organic acids, including formic, malic, citric, acetic, and lactic acids, were quantified through a four-stage process of extraction, filtration, distillation, and titration with standard 0.05 M NaOH (Okolie et al., 2023).

**Microbial fuel cell (MFC) coupling and voltage/current readings**

The preparation of the microbial fuel cell (MFC) involved constructing a salt bridge using a mixture of 2% agar-agar and 1% sodium chloride (NaCl) in a PVC pipe. The anode and cathode chambers were constructed from two 3.5-liter plastic containers, interconnected by the salt bridge. Copper electrodes were utilized for the setup (Hossain et al., 2022). The MFC was coupled by adding 400 g of dry agro-waste and 2500 ml of water to the anode compartment, while the cathode compartment was filled with 2500 ml of water for passive aeration. Voltage and current readings were recorded hourly over a 21-day period after an initial acclimatization phase (Banu et al., 2023). Following this, microbial communities on the electrodes were collected using sterile swabs and cultured on various agar media for identification. A controlled setup was constructed for each of four agro-waste samples, leading to a total of 24 experimental configurations. Readings were taken on a 6-hour interval basis daily for 21 days and the average was taken as the daily readings (Venkataramana et al., 2024).

**Standardization of bacterial/fungal inoculum (McFarland Turbidity Standard) for controlled microbial fuel cell set up**

Four of the microorganisms (*L. fusiformis*, *Bacillus subtilis*, *Geotrichum candidum*, and *Pichia kudriavzevii*) isolated from samples were selected to be re-inoculated into a new set up as single and mixed cultures. The McFarland 0.5 turbidity standard was used to measure the density of bacterial cells and fungal spores (Pinar et al., 2025).

**Physicochemical parameters of agro-waste substrates in controlled Microbial fuel cell**

Over a 21-day period, physicochemical parameters of samples from the anode compartments of controlled MFCs were evaluated at 3-4 day intervals, including temperature, pH, and total titratable acidity (TTA) (Al-Badani et al., 2024). Temperature was measured using a Vici multi-thermometer. pH was determined with a Benchtop pH/mV meter. TTA was assessed by titrating 10 ml of the sample broth with 0.1 M sodium hydroxide (NaOH).

**Statistical analysis**

Data obtained were analyzed by one-way analysis of variance (ANOVA) and means were compared by Duncan's New Multiple Range test (SPSS 23.0). Differences were considered significant at P<0.05. All heat maps are plotted using matplotlib.pyplot (3.2.1) with python coding.

**Results**

**Total microbial load of agro-waste substrates**

Potato peel recorded the highest bacterial count of 3.8×10<sup>4</sup> CFU/ml compared to other agro-wastes examined. Maize husk had the highest fungal load with value of 6.55×10<sup>4</sup> SFU/ml while wheat shaft recorded the lowest fungal load with value of 1.5×10<sup>4</sup> SFU/ml as shown in **Figure 1**.

**Characterization of microbial isolates from agro-waste substrates**

Morphological and biochemical characterization of the bacteria isolated from the agro-wastes as shown in **Table 1** revealed the presence of *Acinetobacter baumannii*, *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Bacillus subtilis*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *Paenibacillus dendritiformis*, *Lactobacillus plantarum*, *Proteus mirabilis*, *Corynebacterium* spp, *Escherichia coli*, *Klebsiella* spp, *Pseudomonas aeruginosa*, *Xanthomonas campestris* and *Enterococcus faecalis*. The yeasts isolated were *Saccharomyces cerevisiae*, *Candida albicans*, *Rhodotorula glutinis* and *Geotrichum candidum* as illustrated in **Table 2**. **Table 3** showed the fungal isolates (moulds) which included; *Aspergillus flavus*, *R. stolonifer*, *A. niger*, *A. terreus*, *Trichophyton mantagrophytes*, *Microsporum canis*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Alternaria alternate*, *Fusarium solani*, and *Neurospora crassa*.

**Table 1: Morphological and biochemical characteristics of isolated bacteria from the agro-waste substrates**

| Isolate code | Colony colour | Gram rxn/ shape | Catalase | Coagulase | Motility | Spore formation | Indole | Starch hydrolysis | Citrate | Urease |
|--------------|---------------|-----------------|----------|-----------|----------|-----------------|--------|-------------------|---------|--------|
| 1            | Creamy        | -ve/ cocci      | +        | -         | -        | +               | +      | +                 | +       | -      |
| 2            | Creamy        | +ve/cocci       | -        | +         | -        | -               | -      | -                 | -       | -      |
| 3            | Creamy        | +ve/cocci       | +        | -         | -        | -               | -      | -                 | +       | -      |
| 4            | Yellow        | +ve/cocci       | +        | -         | -        | -               | -      | -                 | +       | -      |
| 5            | Creamy        | +ve/rod         | +        | -         | +        | +               | -      | +                 | -       | -      |
| 6            | Creamy        | +ve/rod         | +        | -         | +        | +               | -      | +                 | -       | -      |
| 7            | Creamy        | +ve/rod         | +        | -         | +        | +               | -      | +                 | -       | -      |
| 8            | Creamy        | +ve/rod         | +        | -         | +        | +               | -      | +                 | -       | -      |
| 9            | Off white     | +ve/rod         | +        | -         | +        | +               | -      | +                 | +       | -      |
| 10           | Creamy        | +ve/rod         | +        | -         | +        | +               | -      | +                 | +       | -      |
| 11           | Opaque        | +ve/rod         | +        | -         | +        | +               | -      | -                 | -       | -      |
| 12           | Creamy        | -ve/rod         | -        | -         | +        | -               | -      | -                 | -       | +      |
| 13           | Creamy        | -ve/rod         | -        | -         | -        | -               | +      | -                 | -       | -      |
| 14           | Shiny         | -ve/rod         | -        | -         | -        | -               | +      | -                 | +       | -      |
| 15           | Mucoid        | -ve/rod         | -        | -         | -        | -               | -      | -                 | +       | -      |
| 16           | Greenish      | -ve/rod         | -        | -         | -        | -               | -      | -                 | -       | -      |
| 17           | Creamy        | -ve/rod         | -        | -         | -        | -               | -      | -                 | -       | -      |
| 18           | Creamy        | -ve/cocci       | -        | -         | -        | -               | -      | -                 | -       | -      |

Key: += positive, - =negative, A/G= Acid and Gas present, A= acid only

**Table 2: Morphological and biochemical characteristics of yeasts isolated from agro-wastes**

| Isolate code     | Cell shape  | Morphology |                   | Biochemical Properties    |                |          |         |          |         |         |         |     |
|------------------|-------------|------------|-------------------|---------------------------|----------------|----------|---------|----------|---------|---------|---------|-----|
|                  |             |            |                   | Fermentation/Assimilation |                |          |         |          |         |         |         |     |
|                  |             | Ascospore  | Shape             | Spore                     | Pseudomycelium | Mycelium | Glucose | Fructose | Sucrose | Lactose | Maltose | Nit |
| P <sub>3</sub> 3 | Oval        | +          | Oval              | +                         | +              | -        | FA      | FA       | FA      | -A      | FA      | -   |
| P <sub>3</sub> 1 | Cylindrical | +          | Oval              | +                         | +              | -        | FA      | FA       | FA      | --      | FA      | +   |
| P <sub>3</sub> 5 | Oval        | -          | Oval              | -                         | +              | -        | -A      | -A       | -A      | --      | +       | -   |
| S.CA1            | Cylindrical | +          | Short cylindrical | +                         | -              | +        | FA      | FA       | -A      | -A      | FA      | +   |

Key: + = Present, - = Absent, FA= Fermentation and Assimilation, -A= Assimilation

**Table 3: Cultural and morphological characteristics of moulds from the agro-wastes**

| Isolate code | Cultural characteristics                                                                      | Spores/conidia arrangement under the microscope                                     | Identity of isolates               |
|--------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|------------------------------------|
| 1            | Spores are granular, flat, often with radial grooves, yellow                                  | Conidia are globose to sub-globose, pale green                                      | <i>Aspergillus flavus</i>          |
| 2            | Conidia grows rapidly, resemble cotton candy and darken with age                              | Mycelia are marked by numerous stolons connecting groups of long sporangiophores    | <i>Rhizopus stolonifer</i>         |
| 3            | The colonies consist of a compact white with a dense layer of dark brown to black.            | Conidial head, conidiophores are smooth-walled, often in brown colour               | <i>Aspergillus niger</i>           |
| 4            | Pinkish brown mycelia, with a yellow to deep brown reverse.                                   | Conidia are globose to ellipsoidal, hyaline to slightly yellow and smooth walled    | <i>Aspergillus terreus</i>         |
| 5            | Colonies are flat, white in colour, with a powdery surface and downy area.                    | Conidia are marked by numerous single-celled micronidia and multi-celled macronidia | <i>Trichophyton mantagrophytes</i> |
| 7            | Aerial mycelium sparse, whitish with a purple tinge more incense near the medium surface.     | Septate borne on lateral, simple often reduce phialides                             | <i>Fusarium oxysporum</i>          |
| 8            | Fast-growing colonies in green colour. Branching conidiophores, septate and fruiting mycelium | Bluish-green filament is observed which changes to powdery greenish brown.          | <i>Penicillium chrysogenum</i>     |
| 9            | Colonies are fast growing, black to olivaceous black, and are suede-like to floccose          | Branched acropetal chains of multicelled conidia are produced                       | <i>Alternria alternata</i>         |
| 10           | Mycelium grey-white with sparse floccose                                                      | Oval microconidia produced on richly branched conidiophores.                        | <i>Fusarium solani</i>             |
| 11           | Orange coloured broadly spreading colonies                                                    | Ascospores broadly fusiform, nearly spherical, unicellular, to yellowish brown      | <i>Neurospora crassa</i>           |

#### Molecular identification of the dominant microorganisms (bacteria and fungi) from agro-waste substrates

The dominant microorganisms included; *Penicillium* spp, *Geotrichum candidum*, *Lactobacillus* spp, and *Bacillus subtilis*. Deoxyribonucleic acid (DNA) sequencing confirmed that *Pichia kudriavzevii* MN007220.1, *Geotrichum candidum* MK943778.1, *Bacillus subtilis* NR102783.2 and *Lysinibacillus fusiformis* KP419973.1 were elucidated using 16S rDNA analysis as shown in **Table 4**.

**Table 4: Conventional and molecular identification of bacteria and fungi from the agro-waste substrates**

| Name allotted using conventional methods of identification | Name allotted using molecular method of identification | Accession number of sequence with best match | Base pair | Percentage identity |
|------------------------------------------------------------|--------------------------------------------------------|----------------------------------------------|-----------|---------------------|
| <i>Bacillus subtilis</i>                                   | <i>Bacillus subtilis</i>                               | NR102783.2                                   | 1585      | 100                 |
| <i>Lactobacillus</i> spp                                   | <i>Lysinibacillus fusiformis</i>                       | KP419973.1                                   | 1581      | 99.88               |
| <i>Geotrichum candidum</i>                                 | <i>Geotrichum candidum</i>                             | MK943778.1                                   | 500       | 100                 |
| <i>Penicillium</i> spp                                     | <i>Pichia kudriavzevii</i>                             | MN007220.1                                   | 600       | 92.12               |

#### Gel electrophoresis of bacteria and fungi isolated from agro-waste substrates

The electrophoresis gel (**Plate 1**) effectively showcases the identification of yeast microorganisms, specifically *Pichia kudriavzevii* MN007220.1 and *Geotrichum candidum* MK943778.1, within agro-waste substrates through DNA visualization. The gel commences with a molecular ladder in the first lane, featuring DNA fragments of predetermined sizes ranging from 100 bp to 1000 bp (0.1 to 1 kbp). This ladder acts as a vital benchmark, enabling accurate estimation of DNA band sizes in the subsequent sample lanes. *Pichia kudriavzevii* displays a band at approximately 550 bp (0.55 kbp), while *Geotrichum candidum* reveals a band at roughly 750 bp (0.75 kbp). The gel (**Plate 2**) illuminates the detection of bacterial species, namely *Bacillus subtilis* NR102783.2 and *Lysinibacillus fusiformis* KP419973.1, extracted from agro-waste samples via DNA band analysis. Like its yeast counterpart, the gel includes a molecular ladder in the initial lane, with DNA fragments spanning 100 bp to 1000 bp (0.1 to 1 kbp), serving as a critical reference for sizing the bands in adjacent lanes. *Bacillus subtilis* is marked by a band at about 400 bp (0.4 kbp), and *Lysinibacillus fusiformis* is indicated by a band at approximately 650 bp (0.65 kbp).

#### Proximate composition of agro-waste substrates before and after fermentation

Several key trends were observed in the proximate content of the agro-waste substrates before and after fermentation. For Moisture content, the raw substrates have significantly lower percentages compared to the fermented ones, with raw maize husk (D) being the lowest (0.71%) and fermented wheat shaft (C) the highest (38.77%). Ash content generally shows a decrease after fermentation, with raw maize husk (D) having the highest ash (1.86%) and fermented potato peels (A) the lowest (0.49%). Crude protein content mostly increases after fermentation, with raw sugarcane shaft (B) showing the lowest protein (1.49%) and fermented potato peels (A) the highest (14.10%). Crude fat content is generally low across all raw substrates, with raw sugarcane shaft (B) being the lowest (0.04%) and raw maize husk (D) the highest (1.87%); fermentation leads to varying changes in fat content, with fermented sugarcane shaft (B) having the highest fat (2.01%). Crude fibre content tends to decrease after fermentation, with raw maize husk (D) having the highest fibre (16.74%) and fermented wheat shaft (C) the lowest (4.99%). Lastly, Carbohydrate content is highest in the raw substrates, with raw sugarcane shaft (B) showing the highest percentage (84.49%) and fermented wheat shaft (C) the lowest (46.08%), indicating a significant reduction in carbohydrates after fermentation across all substrates as illustrated in **Figure 2**.

#### **The Mineral Content of agro-waste substrates before and after fermentation**

This grouped bar chart (**Figure 3**) effectively elucidates the differential mineral content across various agro-waste substrates before and after fermentation. Potassium (K) exhibits the most substantial concentrations among all analyzed minerals, with wheat shaft (C) demonstrating the preeminent potassium level post-fermentation at approximately 494.00 mg/100 g. Conversely, lead (Pb) consistently presents the lowest concentrations, often at or near zero, both in raw and fermented substrates, indicating its minimal presence. Fermentation generally appears to augment the concentrations of most minerals, notably potassium, highlighting a potential enhancement in nutritional value.

#### **The Vitamin Content in agro-waste substrates before and after fermentation**

The graphical illustration (**Figure 4**) of vitamin content reveals a pronounced disparity in the concentrations of different vitamins within the agro-waste substrates. Vitamin A is present in considerably higher quantities compared to other vitamins, particularly after fermentation. Potato peels (A) exhibit the most elevated Vitamin A content post-fermentation, reaching approximately 828.63 mg/g. In stark contrast, Vitamin B2 generally shows the lowest concentrations across all substrates and treatments. Fermentation demonstrably increases the levels of most vitamins, underscoring its efficacy in enriching the vitamin profile of the agro-wastes.

#### **Enzyme content of agro-waste substrates before and after fermentation**

The most striking observation is the high increase in cellulase content after fermentation across all agro-waste substrates (**Figure 5**). The fermented samples show significantly higher levels of cellulase compared to the raw samples. The highest fermented cellulase content is observed in Maize husk (D) at approximately 9.71 mg/ml/min. The lowest raw cellulase content is also observed in Maize husk (D) at approximately 0.03 mg/ml/min. Amylase content also appears to increase after fermentation, although not as dramatically as cellulase. Potato peels (A) show the highest raw Amylase content at approximately 0.08 mg/ml/min. Potato peels (A) also show the highest fermented Amylase content at approximately 0.24 mg/ml/min. The lowest raw Amylase content is in Maize husk (D) at approximately 0.00 mg/ml/min. The lowest fermented Amylase content is also in Maize husk (D) at approximately 0.01 mg/ml/min. The levels of Protease are relatively low in both raw and fermented samples. The highest raw Protease content is in Potato peels (A), Sugarcane shaft (B), Wheat shaft (C), and Maize husk (D), all at approximately 0.01 mg/ml/min. The highest fermented Protease content is in Wheat shaft (C) and Maize husk (D) at approximately 0.02 mg/ml/min. The lowest raw Protease content is across all samples at approximately 0.01 mg/ml/min. The lowest fermented Protease content is in Potato peels (A) and Sugarcane shaft (B) at approximately 0.01 mg/ml/min. Pectinase content is also relatively low. The highest raw Pectinase content is in Potato peels (A) and Sugarcane shaft (B) at approximately 0.01 mg/ml/min. The highest fermented Pectinase content is in Potato peels (A), Sugarcane shaft (B), and Maize husk (D) at approximately 0.02 mg/ml/min. The lowest raw Pectinase content is in Wheat shaft (C) and Maize husk (D) at approximately 0.00 mg/ml/min. The lowest fermented Pectinase content is in Wheat shaft (C) at approximately 0.01 mg/ml/min.

#### **The Ethanol and Organic Acid Content of agro-waste substrates before and after fermentation**

This grouped bar chart (**Figure 6**) provides a comprehensive overview of the ethanol and organic acid profiles of the agro-waste substrates before and after fermentation. Lactic acid is the predominant organic acid observed, with sugarcane shaft (B) exhibiting the highest concentration post-fermentation at approximately 1.41 mg/L. Ethanol is exclusively detected in fermented substrates, with sugarcane shaft (B) also presenting the highest ethanol yield at approximately 1.16%. Formic acid, malic acids, citric acids, and acetic acids are present in comparatively lower concentrations. The chart clearly outlines the transformative impact of fermentation on the production of these organic compounds.

#### **Voltage Generation by Agro-waste Substrates**

The heat map (**Figure 7**) clearly identifies Maize husk as the top-performing agro-waste substrate for voltage generation, producing the highest voltage of 68 mV facilitated by *Geotrichum candidum* culture. In contrast, Sugarcane shaft exhibited the lowest voltage at 50 mV facilitated by *Lactobacillus fusiformis* culture, marking a notable difference in performance. This superior voltage output from Maize husk is closely tied to its significantly higher bacterial load at the anode electrode compared to the cathode in the microbial fuel cell (MFC). The elevated bacterial activity at the anode drives the electrochemical reactions that generate voltage, creating a greater electrical potential difference between the electrodes. While other substrates, such as Sugarcane and Wheat, also produce substantial voltages, their bacterial and fungal distributions at the electrodes are less optimized than that of Maize husk. The study emphasizes that the bacterial condition at the anode of Maize husk is a critical factor in achieving peak voltage generation, positioning it as a standout substrate for MFC applications. The high voltage generation of 68 mV by Maize husk carries profound implications for the efficiency and viability of MFCs.

#### **Current Generation by Agro-waste Substrates**

According to the heat map (**Figure 8**), Maize husk also excels in current generation, delivering the highest current of 77  $\mu\text{A}$  among the tested agro-waste substrates, facilitated by maize husk-cultured *Geotrichum candidum*. Conversely, sugarcane shaft registers the lowest current at 55  $\mu\text{A}$  facilitated by sugarcane shaft-cultured *Lactobacillus fusiformis*, underscoring Maize husk's superior performance. This elevated current output is directly linked to the substantial bacterial load at the anode electrode of the Maize husk MFC, where bacteria facilitate the transfer of electrons from the substrate to the anode, driving the flow of electrical charge. Although substrates like Sugarcane and Wheat also generate notable currents, their microbial distributions at the electrodes are less effective compared to Maize husk. The study points to the bacterial dynamics at the anode as a pivotal element in achieving peak current generation, reinforcing Maize husk's potential for optimizing MFC systems. The impressive current generation of 77  $\mu\text{A}$  by Maize husk is a vital factor in the practical deployment of MFCs, as current reflects the rate of electrical energy production.

#### Physicochemical Parameters of Agro-waste substrates in Microbial Fuel Cell medium

Maize husk is the top performer, generating the highest voltage (68 mV) and current (77  $\mu\text{A}$ ). This is visually represented by the bright yellow cells, indicating the highest scaled values. In contrast, Potato peels are the least effective, producing the lowest voltage (60 mV) and current (70  $\mu\text{A}$ ). Sugarcane and Wheat shafts show intermediate and comparable electrical outputs. The operational parameters of temperature and pH appear to be correlated with electrical performance. The Maize husk medium sustained the most favorable conditions with the highest temperature (28.5°C) and a near-neutral pH of 6.75. Conversely, the poor electrical output from the Potato peels coincides with a significantly more acidic environment (pH 5.75), which likely inhibited the metabolic activity of the electricity-producing microbes (**Figure 9**)

#### Microbial load of agro-waste substrates at Microbial fuel cell anode and cathode compartments

This heat map (**Figure 10**) illustrates the inferred microbial load (Bacteria and Fungi) at the anode and cathode electrodes for each agro-waste substrate. The color intensity corresponds to the microbial count (CFU/ml for bacteria, SFU/ml for fungi). For sugarcane, specific values are provided: 75 CFU/ml bacteria at the cathode and 0 at the anode, and 15 SFU/ml fungi at the cathode and 65 at the anode. This clearly shows a high bacterial load at the sugarcane cathode and a high fungal load at the sugarcane anode. Bacterial load at the anode was significantly greater than at the cathode for potato and maize, and fungal load at the sugarcane anode was significantly greater than at the cathode. This indicates the microbial distribution, emphasizing the differential colonization patterns on the electrodes depending on the agro-waste substrate and microbe type.

#### Physicochemical metrics of Controlled Microbial Fuel Cell (MFC) by agro-waste substrates

The heat map (**Figure 11**) provides a comparative view of three physicochemical properties across the four agro-waste substrates (A: Potato peels, B: Sugarcane shaft, C: Wheat shaft, D: Maize husk). The heat map shows that the temperature is relatively consistent across all agro-waste substrates, ranging from approximately 26.0°C (Potato peels - A) to 27.5°C (Maize husk - D). The temperature is within the mesophilic range (24–27°C or 26.5–29.5°C) with no significant difference between agro-wastes. This consistency in temperature suggests that the different substrates themselves do not drastically alter the temperature of the MFC medium, and the operational temperature is likely suitable for microbial activity in general. The pH values show more variation across the agro-wastes. The heat map clearly indicates that Potato peels (A) have the lowest pH at 5.75, while Maize husk (D) has the highest pH at 6.75. The pH of the medium is a critical factor for microbial growth and activity in MFCs. The varying pH values suggest that each agro-waste creates a slightly different chemical environment, which could favor different microbial communities and thus impact MFC performance. The heat map shows an inverse relationship between pH and TTA, as mentioned in the text. Potato peels (A), with the lowest pH, have the highest TTA at 0.8, while Maize husk (D), with the highest pH, has the lowest TTA at 0.3. TTA is an indicator of the total amount of acidic substances in the medium, often including organic acids produced during microbial metabolism. Higher TTA generally corresponds to a more acidic environment (lower pH). The variation in TTA suggests different levels of organic acid production or consumption by the microbial communities associated with each agro-waste. The pH and Total Titratable Acidity (TTA) appear to be the most variable parameters among those in the current heat map and are known to significantly influence the microbial activity that drives voltage and current generation. While temperature is important, its consistency across substrates. The higher pH and lower TTA observed in Maize husk (D), suggest that these conditions might be more favorable for the specific microbes responsible for efficient electron transfer and power generation in this MFC setup compared to the lower pH and higher TTA found in substrates like Potato peels (A). pH and TTA are the most significant physicochemical parameters shown in this heat map that differentiate the agro-wastes and likely contribute to the varying MFC outputs.

#### Occurrence of microorganisms in the agro-wastes, microbial fuel cell and the electrode of microbial fuel cell

**Table 5** reveals distinct patterns of microbial occurrence across agro-wastes and MFC environments, with some organisms thriving broadly while others are more restricted. Among the most occurring organisms, *Bacillus subtilis* (bacteria) and the fungi *Fusarium solani*, *Fusarium oxysporum*, *Microsporum canis*, and *Penicillium chrysogenum* stand out, being present in all four conditions—agro-wastes, after MFC, anode, and cathode—indicating their robust adaptability and likely key roles in the MFC system, possibly contributing to biofilm formation or electron transfer at the electrodes. Other bacteria like *Acinetobacter baumannii*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *Corynebacterium* spp, *Enterococcus faecalis*, *Lactobacillus plantarum*, *Proteus mirabilis*, and *Staphylococcus aureus* are highly prevalent, present in agro-wastes, after MFC, and at the anode, but absent at the cathode, suggesting a preference for anode-specific conditions, perhaps tied to anodic respiration. Conversely, the least occurring organisms, such as *Klebsiella* spp, *Paenibacillus dendritiformis*, *S. epidermidis*, *Candida albicans*, *Saccharomyces cerevisiae*, *Rhodotorula glutinis*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Neurospora crassa*, *Rhizopus stolonifer*, and *Trichophyton mentagrophytes*, are detected only in agro-wastes and absent in all MFC-related conditions (after MFC, anode, and cathode), indicating poor survival or competitiveness post-MFC operation, possibly due to environmental shifts like pH, oxygen levels, or substrate changes. Organisms like *Pseudomonas aeruginosa*, absent in agro-wastes but present after MFC and at the cathode, highlight

niche specialization, thriving in cathode-specific conditions, potentially linked to oxygen availability. This underscores a microbial hierarchy where resilient species dominate MFC dynamics, while less adaptable ones are confined to the initial agro-waste phase.

**Table 5: Occurrence of Microorganisms in Agro-wastes and MFC Set up**

| Organism                            | Agro-wastes | After MFC | Anode | Cathode |
|-------------------------------------|-------------|-----------|-------|---------|
| <b>Bacteria</b>                     |             |           |       |         |
| <i>Acinetobacter baumannii</i>      | +           | +         | +     | -       |
| <i>Bacillus subtilis</i>            | +           | +         | +     | +       |
| <i>B. cereus</i>                    | +           | +         | +     | -       |
| <i>B. licheniformis</i>             | +           | +         | +     | -       |
| <i>B. megaterium</i>                | +           | +         | +     | -       |
| <i>Corynebacterium spp</i>          | +           | +         | +     | -       |
| <i>Escherichia coli</i>             | +           | +         | -     | +       |
| <i>Enterococcus faecalis</i>        | +           | +         | +     | -       |
| <i>Klebsiella spp</i>               | +           | -         | -     | -       |
| <i>Lactobacillus plantarum</i>      | +           | +         | +     | -       |
| <i>Micrococcus luteus</i>           | +           | +         | -     | +       |
| <i>Paenibacillus dendritiformis</i> | +           | -         | -     | -       |
| <i>Proteus mirabilis</i>            | +           | +         | +     | -       |
| <i>Pseudomonas aeruginosa</i>       | -           | +         | -     | +       |
| <i>Staphylococcus aureus</i>        | +           | +         | +     | -       |
| <i>S. epidermidis</i>               | +           | -         | -     | -       |
| <i>Xanthomonas campestris</i>       | +           | +         | -     | +       |
| <b>Yeasts</b>                       |             |           |       |         |
| <i>Candida albicans</i>             | +           | -         | -     | -       |
| <i>Geotrichum candidum</i>          | +           | +         | +     | -       |
| <i>Saccharomyces cerevisiae</i>     | +           | -         | -     | -       |
| <i>Rhodotorula glutinis</i>         | +           | -         | -     | -       |
| <b>Fungi</b>                        |             |           |       |         |
| <i>Alternaria alternata</i>         | +           | -         | -     | -       |
| <i>Aspergillus niger</i>            | +           | -         | -     | -       |
| <i>Aspergillus flavus</i>           | +           | -         | -     | -       |
| <i>Aspergillus terreus</i>          | +           | -         | -     | -       |
| <i>Fusarium solani</i>              | +           | +         | +     | +       |
| <i>Fusarium oxysporum</i>           | +           | +         | +     | +       |
| <i>Microsporum canis</i>            | +           | +         | +     | +       |
| <i>Neurospora crassa</i>            | +           | -         | -     | -       |
| <i>Penicillium chrysogenum</i>      | +           | +         | +     | +       |
| <i>Rhizopus stolonifer</i>          | +           | -         | -     | -       |
| <i>Trichophyton mentagrophytes</i>  | +           | -         | -     | -       |

**Key:** + = present, - = absent

## Discussion

The initial microbial loads observed in the raw agro-wastes provide a clear illustration of substrate-driven ecological selection. Potato peel, which is rich in readily available starch and other simple carbohydrates, recorded the highest bacterial count (Olorunnisola and Onwudili, 2024; Pinar et al., 2025). This environment favors rapid colonization by heterotrophic bacteria capable of swift substrate turnover. Conversely, maize husk, characterized by its high content of recalcitrant lignocellulose, exhibited the highest fungal load (Guldhe et al., 2023; Ban et al., 2024). This finding is consistent with the well-established role of filamentous fungi as primary decomposers of complex plant biomass, owing to their ability to secrete a powerful arsenal of cellulolytic and lignolytic enzymes (Guldhe et al., 2023).

The subsequent identification of *Pichia kudriavzevii*, *Geotrichum candidum*, *Bacillus subtilis*, and *Lysinibacillus fusiformis* as the dominant microorganisms post-fermentation is not coincidental. Rather, it reflects the selection of robust and metabolically versatile species well-adapted to the specific physicochemical conditions of the fermentation and MFC environments. For instance, *P. kudriavzevii* is a non-conventional yeast renowned for its exceptional tolerance to a wide range of environmental stressors, including low pH, high temperatures, and the presence of fermentation inhibitors like organic acids—conditions that are characteristic of MFC anolytes (Chu et al., 2023; Zvonareva et al., 2024; Okane et al., 2025). Similarly, *G. candidum* is a potent producer of cellulases and other hydrolytic enzymes, making it highly competitive in lignocellulose-rich substrates (Kamilari et al., 2023). The prevalence of spore-forming bacteria from the genera *Bacillus* and *Lysinibacillus* further points to the selection of resilient organisms capable of withstanding fluctuating environmental conditions while contributing to biomass degradation (Liu et al., 2024; Oladipo et al., 2024). The dominance of this particular consortium is therefore a logical outcome of their combined hydrolytic capabilities and environmental resilience.

The significant shifts in the proximate composition of the agro-wastes following fermentation directly reflect the metabolic activities of the dominant microbial consortium. The observed increase in crude protein content is a hallmark of converting low-value biomass into high-quality single-cell protein (SCP) (Eze et al., 2025; Ji et al., 2025). This enrichment is primarily due to the proliferation of microbial biomass, which is itself protein-rich (Bergman and Pandhi, 2023). Fungi and yeasts, such as the identified *G. candidum* and *P. kudriavzevii*, are particularly efficient producers of SCP from agricultural residues (Elhalis et al., 2021; Lu et al., 2025). This finding aligns with a growing body of research focused on using fermentation to produce sustainable, alternative protein sources for animal feed, thereby reducing dependence on conventional crops (Sileshi et al., 2025; Eze et al., 2025).

The concurrent reduction in crude fiber and carbohydrate content is a direct consequence of enzymatic hydrolysis. The breakdown of complex polysaccharides like cellulose and hemicellulose into simpler sugars is driven by the extracellular enzymes secreted by the microbial community, particularly the fungal isolate *G. candidum* and the bacterial isolates *B. subtilis* and *L. fusiformis* (Guldhe et al., 2023; Kamilari et al., 2023; Carranza-Méndez et al., 2025). These sugars are then consumed by the microorganisms for growth and metabolism, leading to the observed decrease in total carbohydrates. This process not only liberates energy for the microbial consortium but also fundamentally improves the digestibility and nutritional availability of the substrate for livestock (Nath et al., 2024; Carranza-Méndez et al., 2025). Furthermore, the significant increase in vitamin content post-fermentation can be attributed to the *de novo* synthesis of essential vitamins by the fermenting microorganisms, a recognized benefit of microbial processing that enhances the overall nutritional profile of the final product (Eze et al., 2025; Ji et al., 2025).

The superior voltage and current generation from the maize husk substrate in the MFC can be explained by a clear, substrate-driven biochemical cascade. As established, the high lignocellulose content of maize husk selected for a dominant fungal community, likely led by the potent cellulase producer *Geotrichum candidum* (Guldhe et al., 2023; Kamilari et al., 2023). This efficient primary degradation of complex polysaccharides would have resulted in a higher and more sustained release of fermentable sugars (e.g., glucose, xylose) compared to the other substrates. These sugars were subsequently fermented into volatile fatty acids (VFAs), which are the preferred fuel for most exo-electrogenic bacteria. This enhanced availability of electron donors at the anode directly fueled a higher rate of microbial respiration and, consequently, greater electron flux to the electrode, manifesting as higher voltage and current.

This interpretation is strongly supported by the observation of a significantly higher microbial load at the anode of the maize husk MFC. The performance of an MFC is intrinsically linked to the density, viability, and metabolic activity of the anodic biofilm (Hossain et al., 2022; Al-Badani et al., 2024). A denser biofilm provides a larger number of catalytic sites for substrate oxidation and facilitates more efficient interspecies electron transfer, ultimately boosting power density (Hossain et al., 2022). The favorable physicochemical conditions within the maize husk medium, particularly the near-neutral pH (6.75), likely created an optimal environment for the proliferation and metabolic activity of the exo-electrogenic members of the consortium, further contributing to its superior performance. In contrast, the more acidic environment (pH 5.75) in the potato peel medium may have inhibited key enzymatic activities or the growth of sensitive exo-electrogens, leading to its comparatively lower electrical output.

*Bacillus subtilis* is a well-documented and robust exo-electrogen, frequently employed in MFCs for its efficient electron transfer capabilities and metabolic versatility (Wang et al., 2021; Mohammed et al., 2024; Liu et al., 2024). Its presence provides a strong foundation for bioelectricity generation within the consortium. *Lysinibacillus fusiformis* represents a more novel but highly promising electrogenic bacterium. Its strong performance on the wheat substrate is a notable finding. Recent research highlights its capacity for degrading complex pollutants and its emerging application in MFCs, suggesting it is an efficient biocatalyst for both bioremediation and power generation (Mei et al., 2022; Oladipo et al., 2024; Hooi et al., 2025). Its ability to produce cellulases further enhances its role in breaking down fibrous substrates. *Pichia kudriavzevii*, while its direct electrogenic activity is less studied than bacterial counterparts, likely plays a critical stabilizing role. Its exceptional tolerance to the acidic and inhibitor-rich conditions common in MFCs allows it to thrive where other microorganisms might be inhibited (Chu et al., 2023; Zvonareva et al., 2024). Furthermore, related *Pichia* species have demonstrated electrogenic potential, suggesting it may contribute directly to current generation while also fermenting a wide range of sugars to produce VFAs for its bacterial partners (Guldhe et al., 2023). *Geotrichum candidum* serves as the consortium's primary hydrolytic powerhouse. Its main contribution is the enzymatic breakdown of complex lignocellulose, initiating the entire metabolic cascade by providing fermentable sugars (Kamilari et al., 2023). Its additional value lies in its established use as a probiotic and a source of SCP, which directly reinforces the application of the fermented substrate as a high-quality animal feed (Lu et al., 2025; Gohar et al., 2025).

The robust performance of the mixed cultures suggests the presence of powerful synergistic interactions. A temporal synergy may be at play, mirroring the dynamics of two-stage SSF, where the initial hydrolytic activity of fungi like *G. candidum* creates an acidic environment and liberates simple substrates that are then efficiently utilized by the more acid-tolerant yeast (*P. kudriavzevii*) and exo-electrogenic bacteria (*B. subtilis*, *L. fusiformis*) (Yan et al., 2025). Furthermore, metabolic synergies, such as the production of riboflavin (an electron shuttle) by *Bacillus* species, which can be utilized by yeasts to enhance electron transfer, may also be occurring, leading to an overall system efficiency greater than the sum of its parts (Wang et al., 2021).

This study successfully demonstrates a proof-of-concept for the dual-purpose valorization of common agro-wastes using their own indigenous microbial consortia. The findings underscore that these waste streams are not merely burdens for disposal but are rich reservoirs of robust, functionally adapted microorganisms capable of driving a synergistic biorefinery process. This integrated approach, which simultaneously produces enhanced-protein animal feed and bioelectricity, offers a tangible pathway toward more sustainable and circular agricultural systems. By converting waste into valuable products, this strategy can reduce feed costs, generate renewable energy on-site, and minimize the environmental footprint of farming operations (Nath et al., 2024).

## Conclusions

The findings from the microbial fuel cell set up showed that Maize husk recorded the highest voltage and current as compared to other agro wastes. Higher microbial load was recorded at the anode as compared to the cathode electrode for all the agro wastes substrate of the MFC set up. Some microorganisms isolated from the agro wastes were also found in the MFC set up, while there were also microbes found in the MFC set up but, not isolated from the agro wastes. The variation observed in voltage production from the controlled MFC set up could be attributed to the type of agro waste used as substrate and the utilizing microorganisms. The findings from this study showed that *Pichia kudriavzevii* MN007220.1, *Geotrichum candidum* MK943778.1, *Bacillus subtilis* NR102783.2 and *Lysinibacillus fusiformis* KP419973.1 were the dominant microorganisms responsible for the degradation of the agro wastes which could have potential biotechnological applications. Hence, it can be recommended as good supplement in compounding animal feed provided that it is acceptable and highly digestible. Furthermore, the study also showed that agro wastes can be implemented as a suitable substrate for bioelectricity production.

## Prospective Research

To advance this technology toward practical application, several future research directions are warranted. A comprehensive metagenomic and metatranscriptomic analysis of the microbial consortia would provide deeper insights into the specific metabolic pathways and gene expression profiles responsible for hydrolysis and electron transfer. This knowledge could inform strategies for targeted consortium engineering to further enhance efficiency. Secondly, optimization of the MFC reactor design and operational parameters (substrate loading rate, hydraulic retention time) is necessary to maximize power output and process stability for these specific agro-waste feedstocks. Finally, conducting comprehensive animal feeding trials is crucial to validate the nutritional value, digestibility, and safety of the fermented substrates, confirming their suitability as a sustainable component of livestock diets.

## Abbreviations

NaOH – Sodium hydroxide

HCl – Hydrochloric acid

H<sub>2</sub>SO<sub>4</sub> – Sulphuric acid

AAS - Atomic absorption spectrophotometer

EDTA - Ethylene-diaminetetraacetic acid

PCA - Perchloric acid

TCA - Trichloroacetic acid

PVC - Polyvinyl chloride

BaCl<sub>2</sub>·2H<sub>2</sub>O - Barium chloride solution

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

All data generated or analysed during this study are included in this published article.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

**GOA:** Performed the experiments; data analysis and interpretation; wrote the first draft. **DJA:** Conceived and supervised the experiments. **MTB:** Proofread the paper, review, editing, conduct data analysis and visualization. All authors read and approved the final manuscript.

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## Plate

Plate 1 and 2 are available in the Supplementary Files section.

## Figures

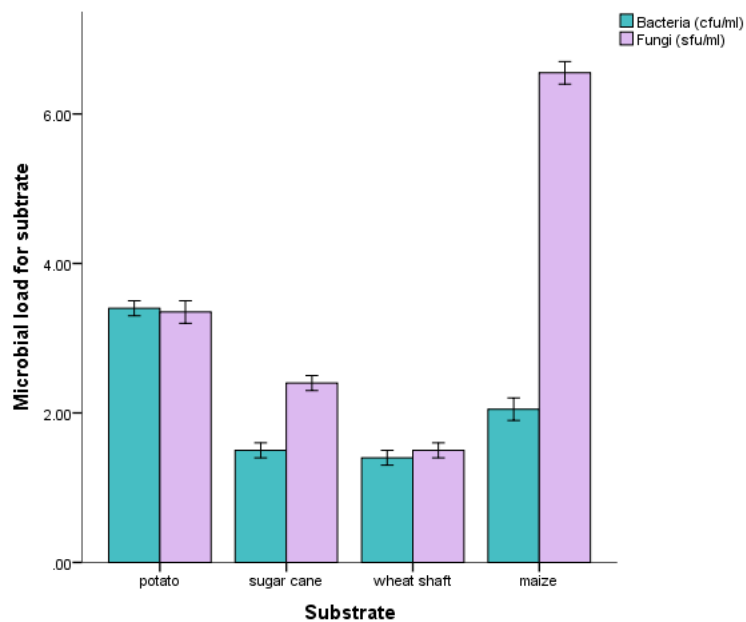


Figure 1

Microbial load ( $\times 10^4$ ) of substrates

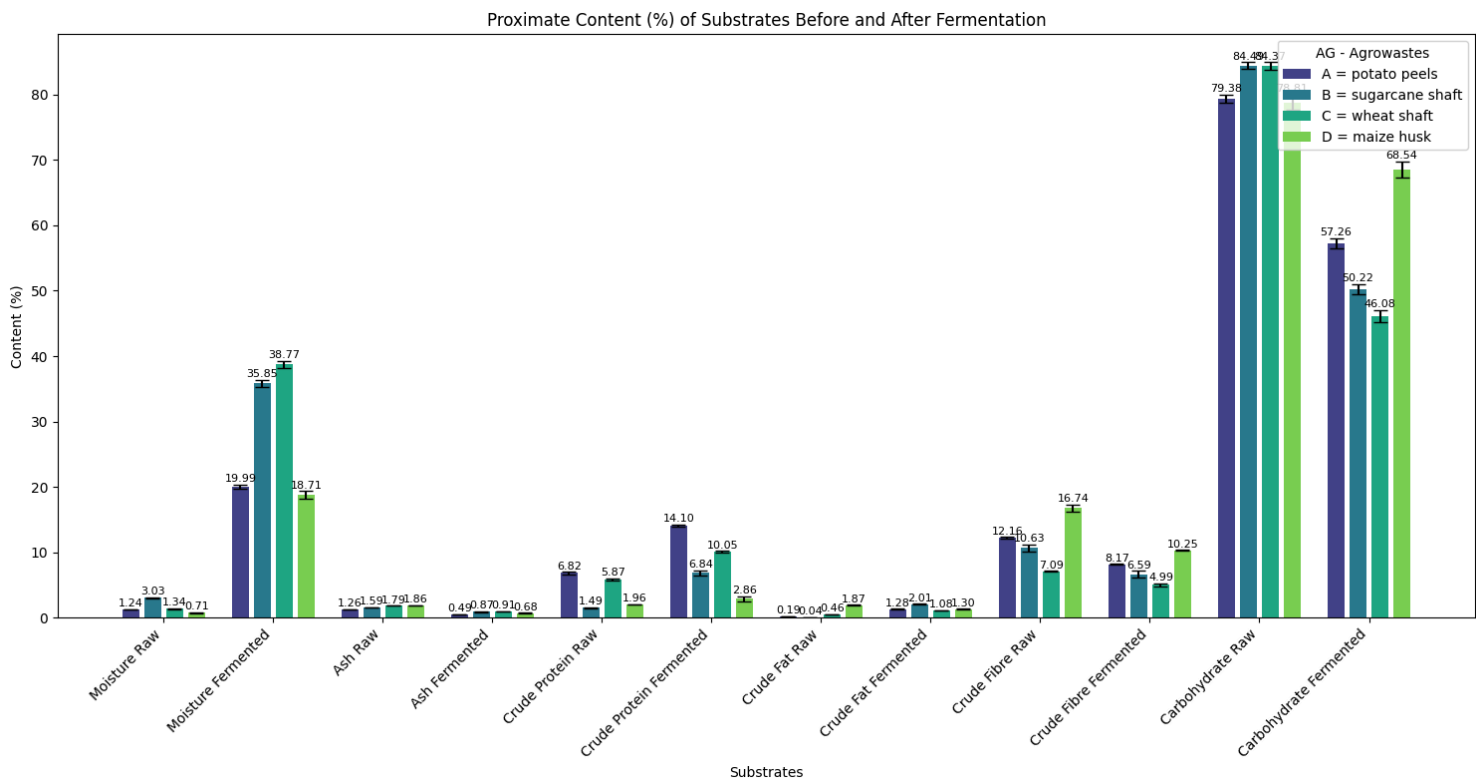


Figure 2

Percentage (%) Proximate Content of substrates in Agro-wastes before after Fermentation

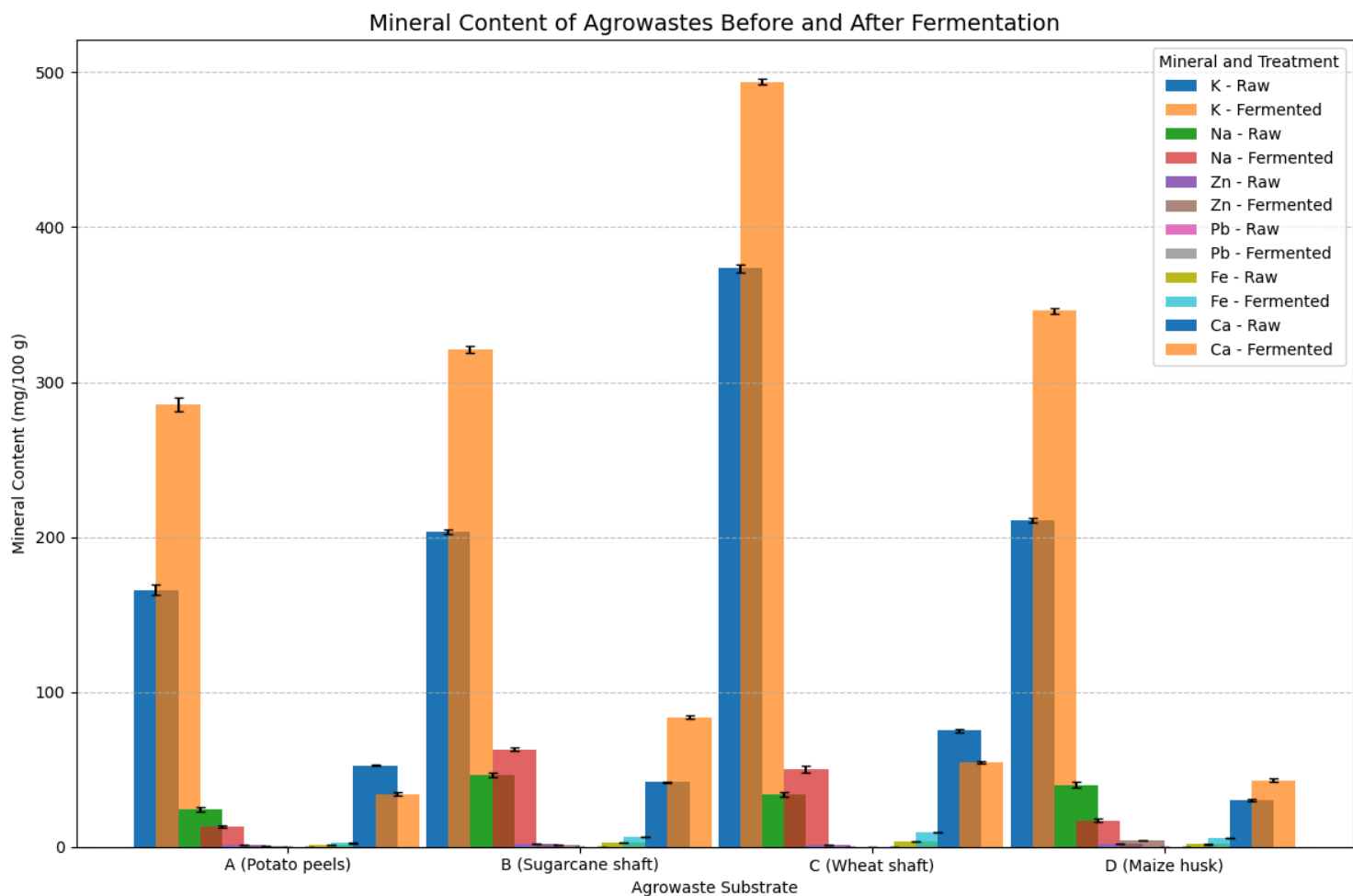


Figure 3

The mineral content (mg/100g) of agro-wastes before and after fermentation

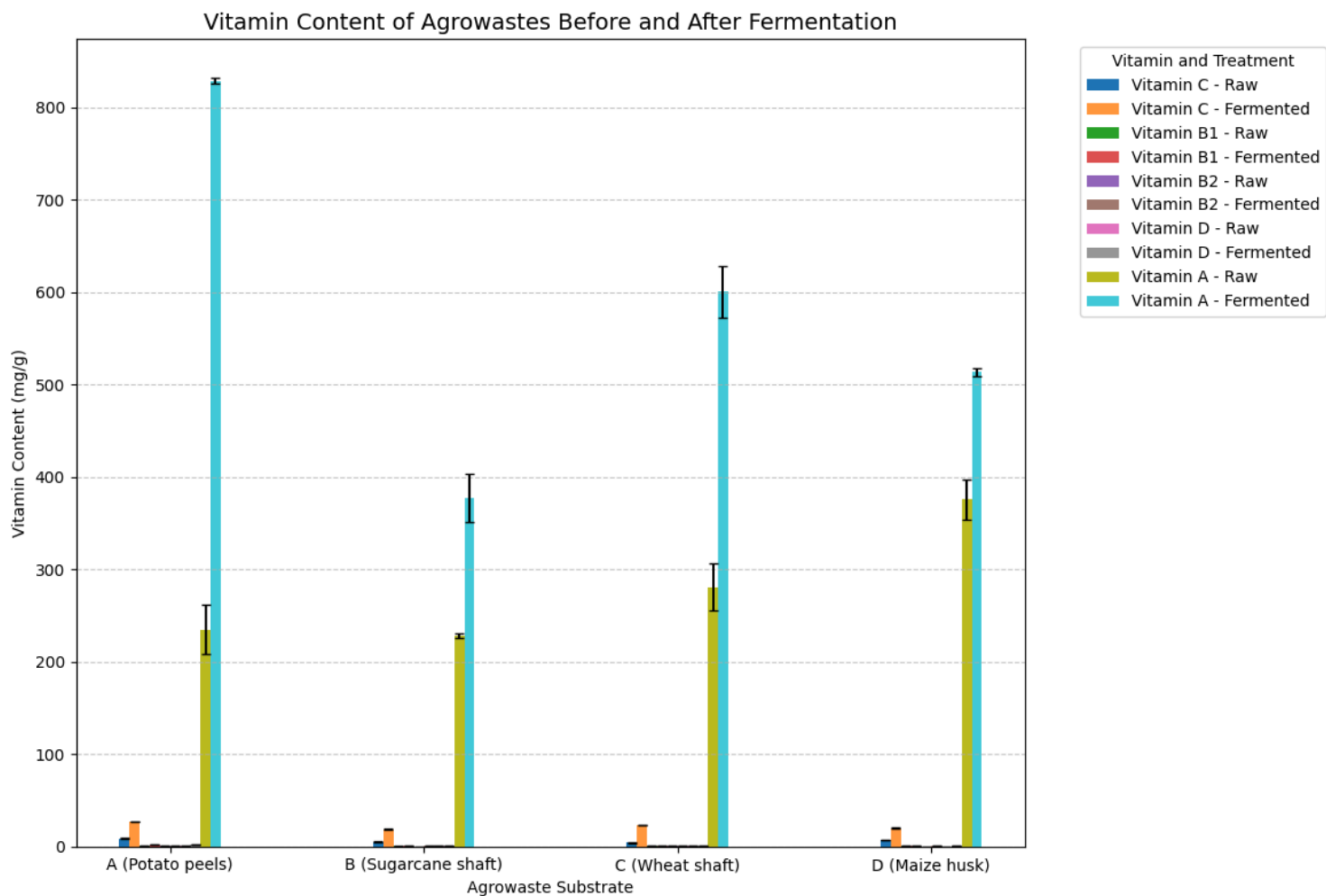


**Figure 4**  
 The Vitamin content (mg/g) of agro-wastes before and after fermentation

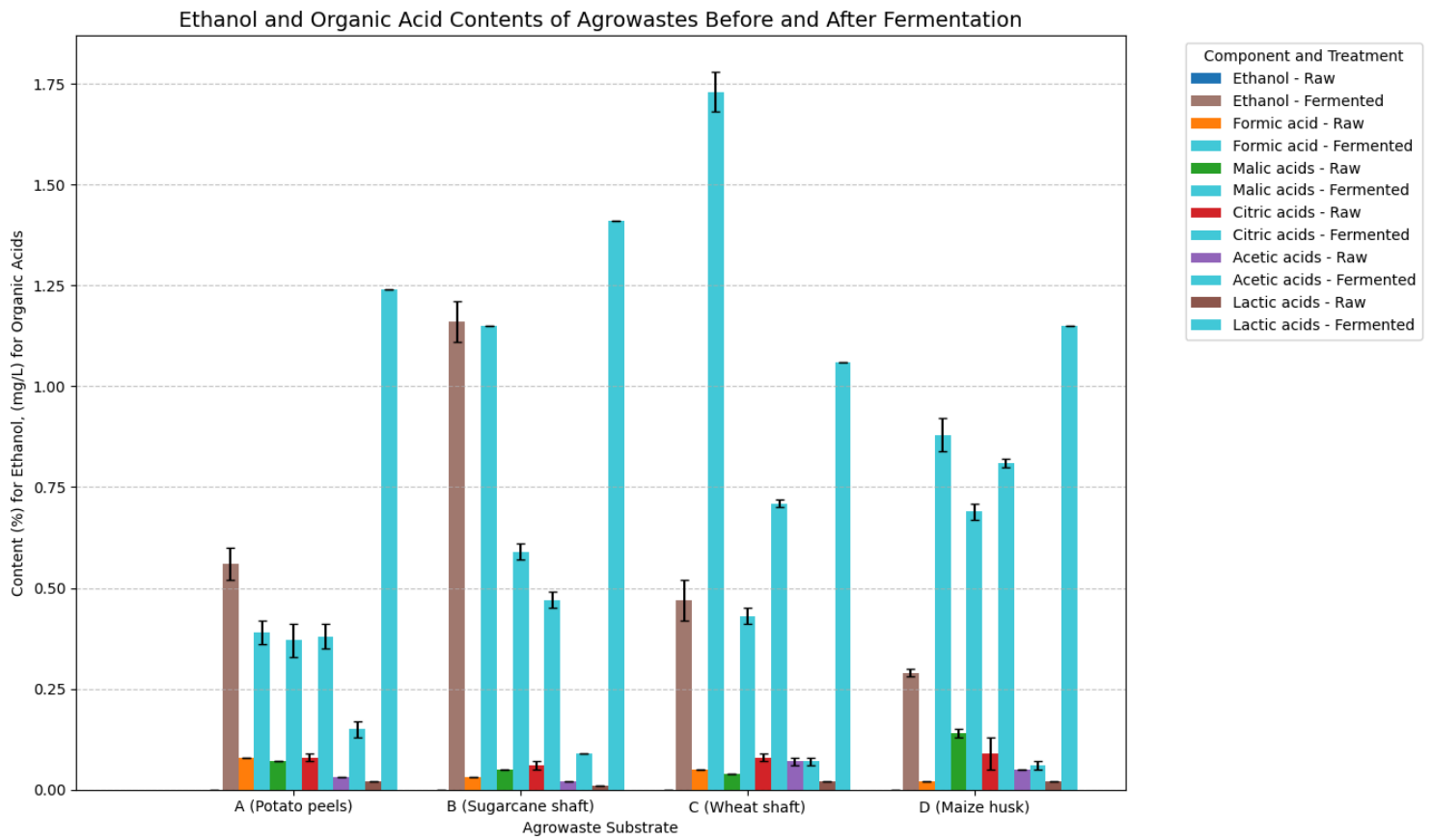


**Figure 5**

Ethanol and Organic Acid Contents of Agro-wastes before and after Fermentation

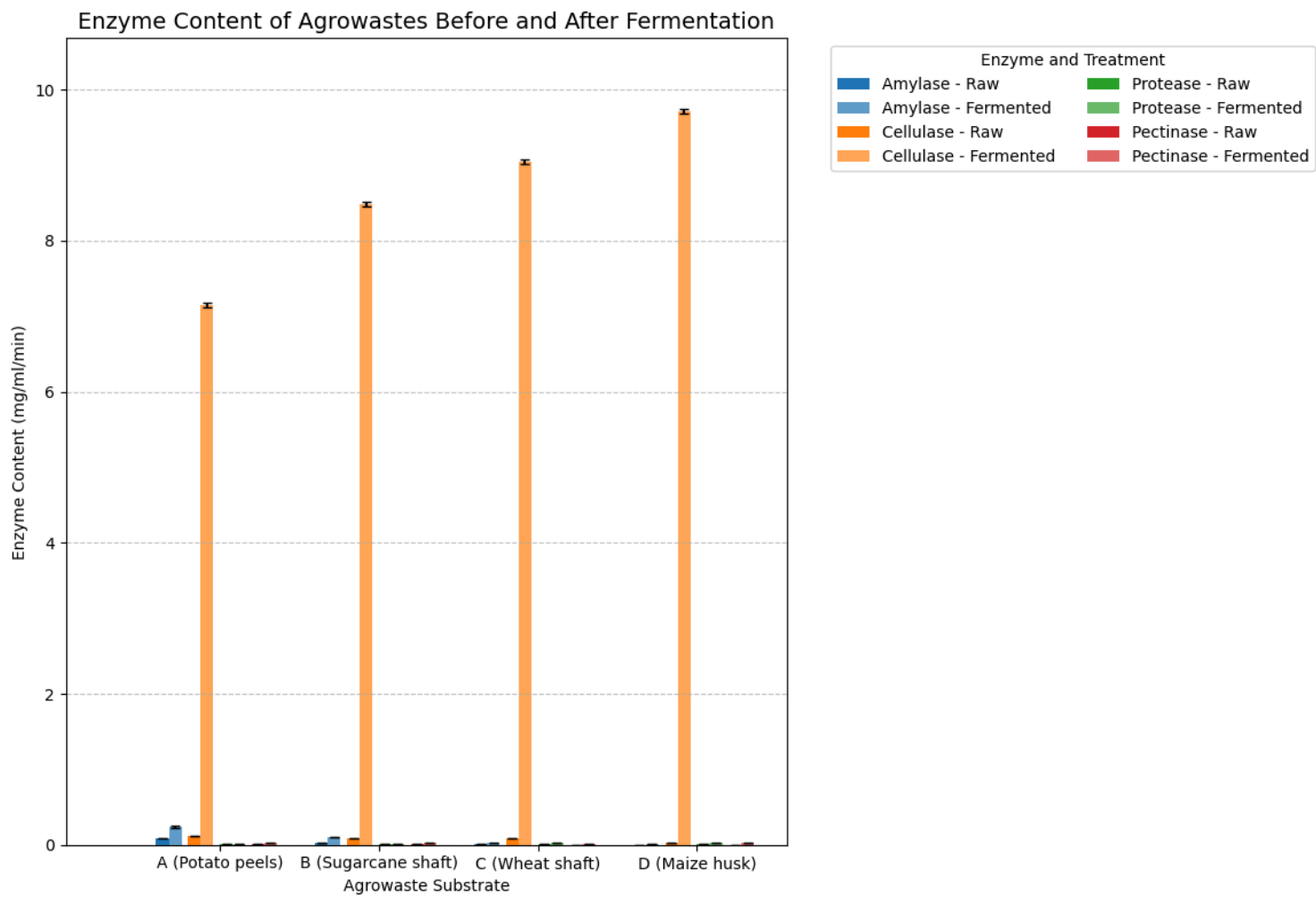


Figure 6

Enzymatic composition (mg/ml/min) agro-waste substrates before and after fermentation

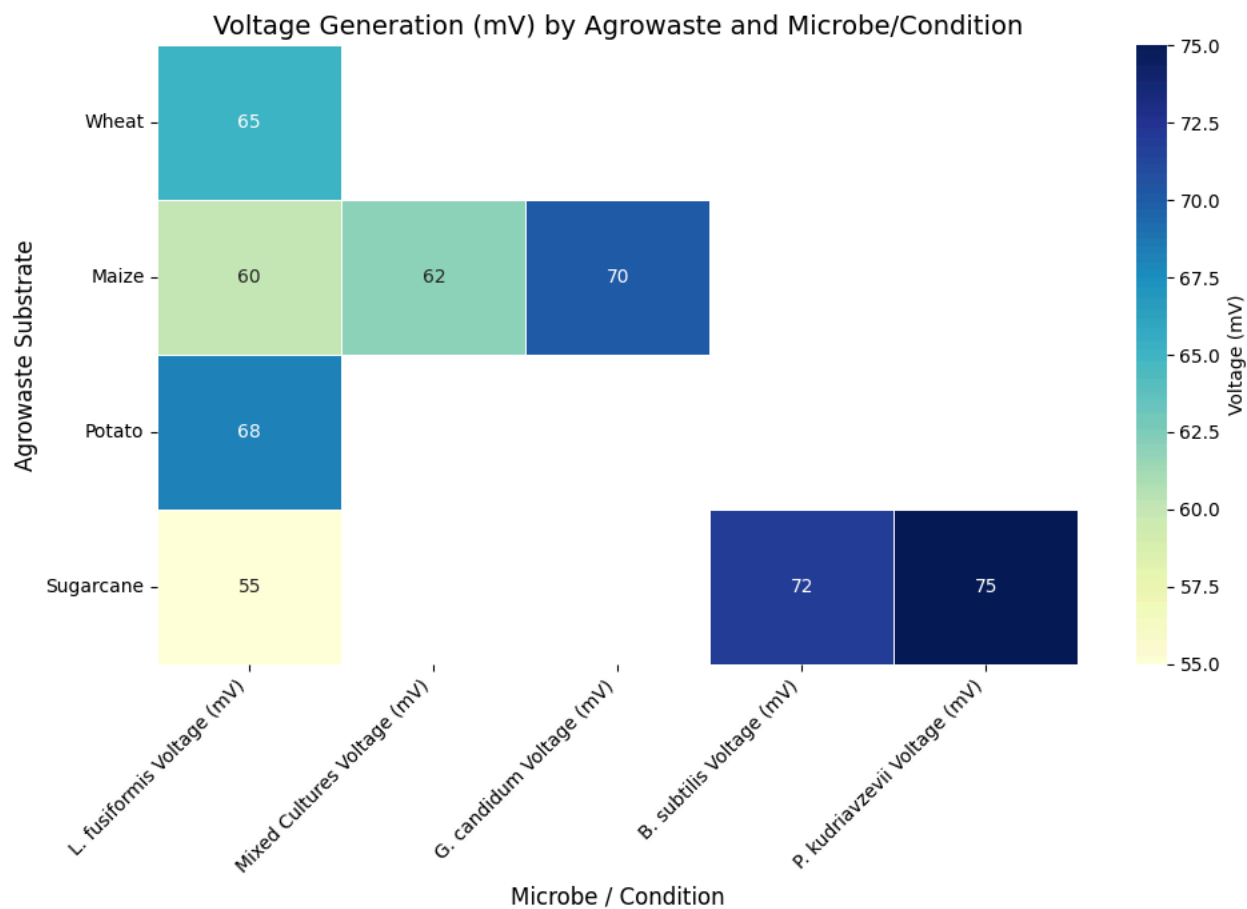


Figure 7

Voltage generation (mV) by Agro-waste substrate-cultured bacteria and fungi

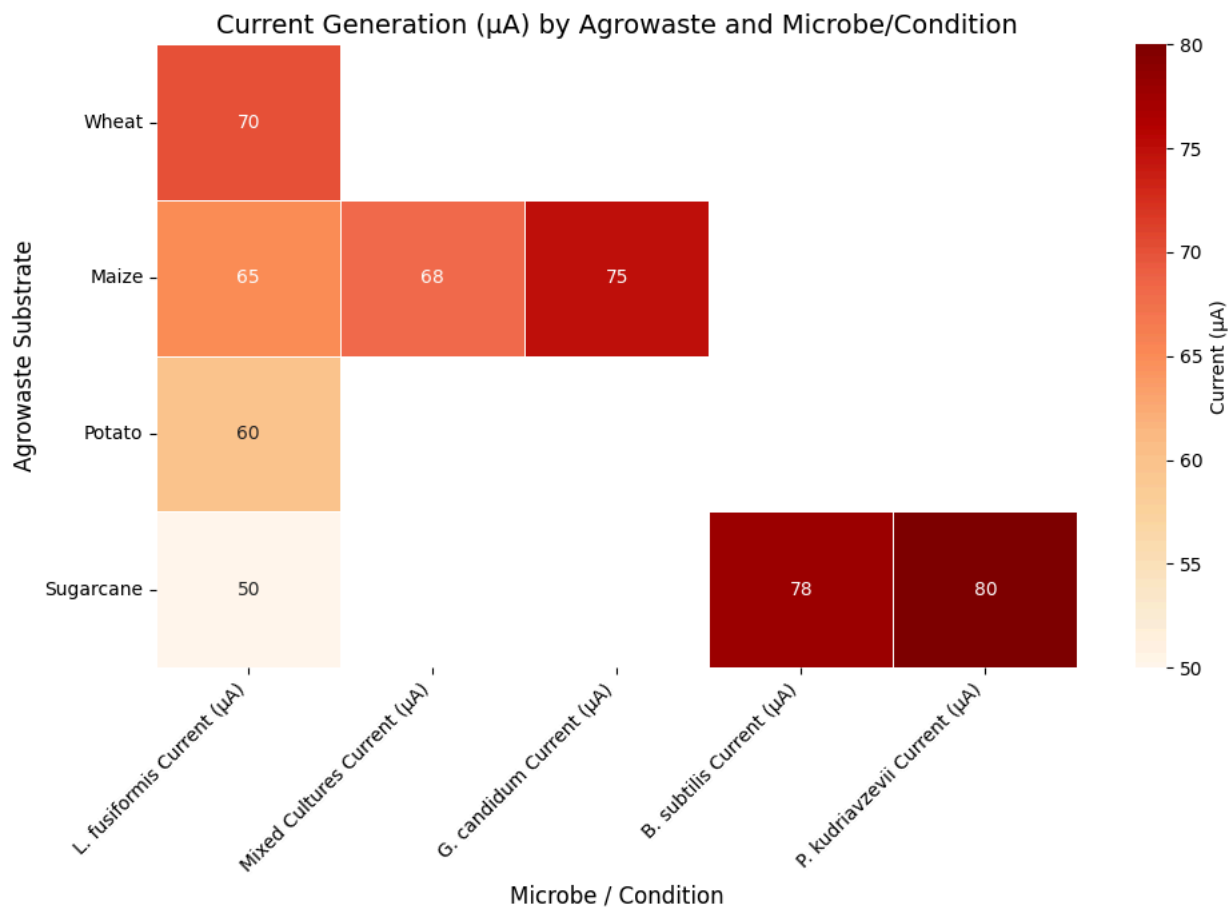


Figure 8

Current generation ( $\mu\text{A}$ ) by Agro-waste substrate-cultured bacteria and fungi

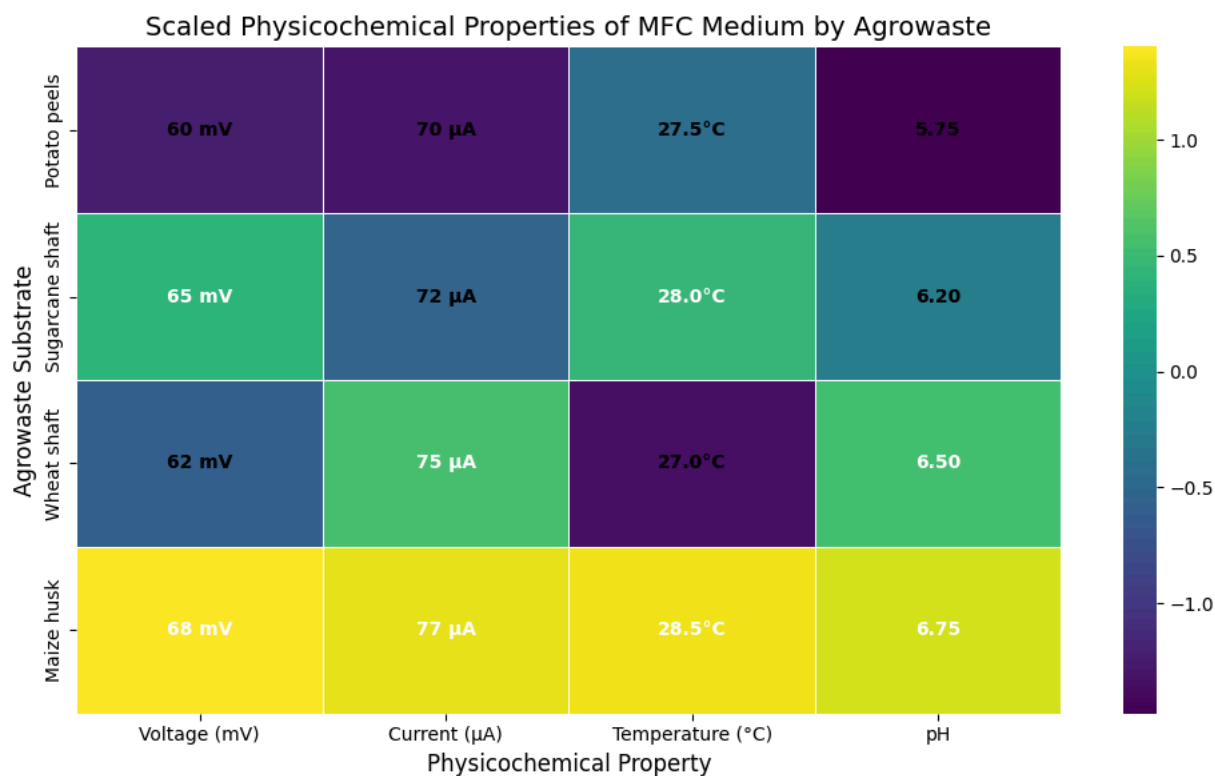


Figure 9

Physicochemical parameters of agro-waste substrates in Microbial fuel cell (MFC) medium

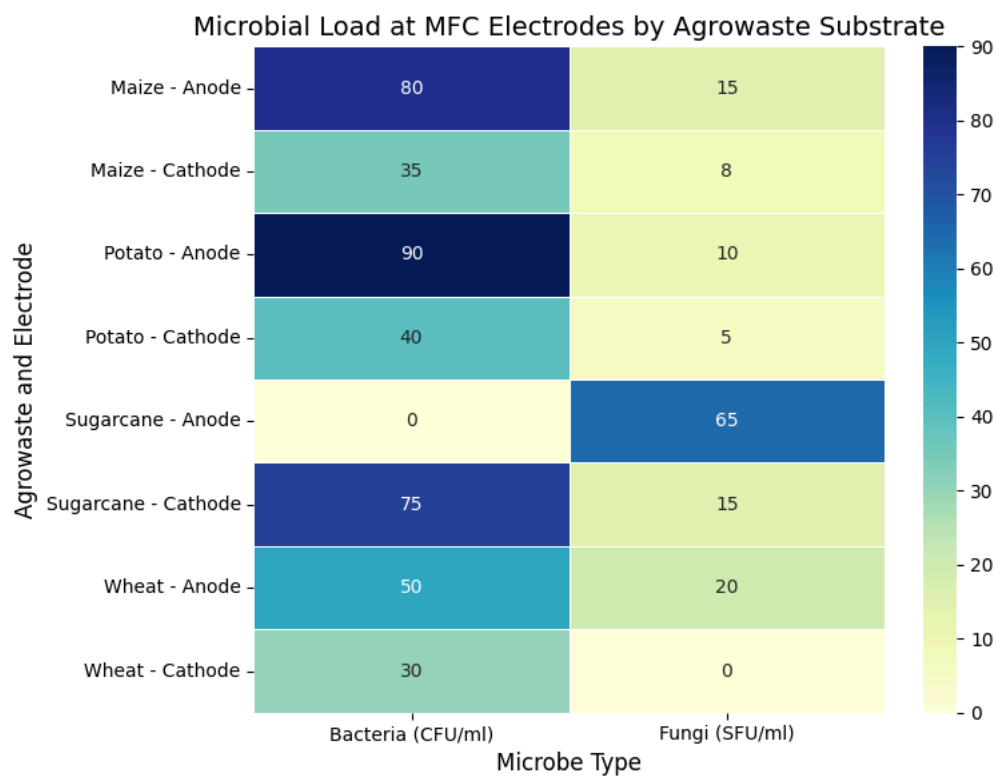


Figure 10

Microbial load of agro-waste substrates at Microbial fuel cell (MFC) Electrodes compartments of anode and cathode

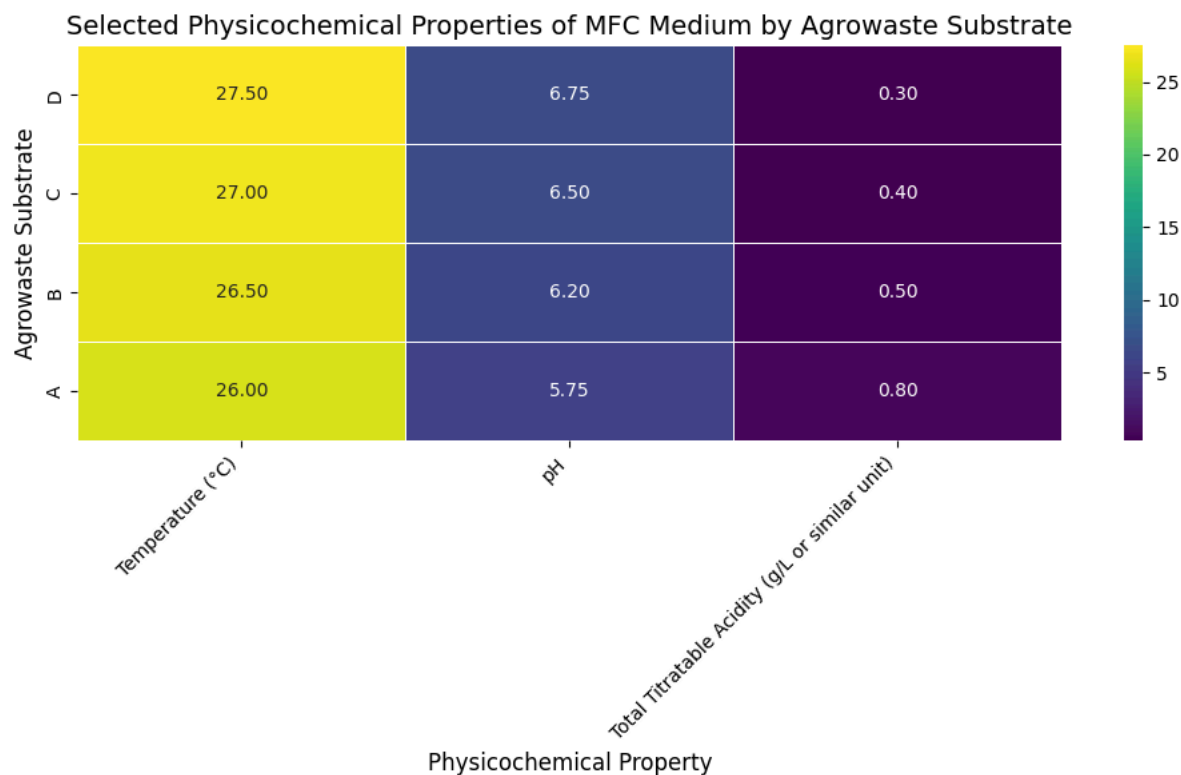


Figure 11

Physicochemical metrics of Controlled Microbial Fuel Cell (MFC) by agro-waste substrates

Key: A- Maize husk; B- Wheat shaft; C- Sugarcane shaft; D- Potato peels

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [MFCagrowastesupplementaryfile.docx](#)
- [GraphicalAbstract.docx](#)
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