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Valorization of Cassava Peel Waste for Sustainable Electricity Generation in a Bacillus subtilis-Inoculated Double-Chamber Microbial Fuel Cell

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Microbial fuel cells (MFCs) provide a sustainable approach for simultaneous waste valorization and bioelectricity production through the metabolic activities of electrogenic microorganisms. This study evaluated bioelectricity generation from cassava peel substrate inoculated with *Bacillus subtilis* using different substrate concentrations (7.5–30 g) over a 14-day period. *Bacillus subtilis* functions as an electrogen by oxidizing organic substrates and transferring electrons generated during metabolism to the anode surface

through extracellular electron transfer mechanisms, thereby facilitating current generation. Morphological characterization of the isolate showed creamy white colonies with irregular margins, rod-shaped Gram-positive cells occurring singly or in chains, while biochemical tests revealed positive reactions for catalase, citrate utilization, starch hydrolysis, and motility, confirming the identity of *Bacillus subtilis*. Voltage output varied with substrate concentration, with peak voltages of 0.49 V, 0.089 V, 0.70 V, and 0.73 V recorded for 7.5 g, 15 g, 22.5 g, and 30 g treatments respectively. Maximum power outputs were 0.0952 W (7.5 g) and 0.0182 W (22.5 g), while current generation reached 0.28 A and 0.20 A respectively. Power density increased progressively, reaching 17.856 W/m^2 at the highest substrate concentration, indicating enhanced electrochemical performance with increasing cassava peel loading.

Keywords: Bacillus subtilis; microbial fuel cell; cassava peel; bioelectricity; electrogenic bacteria; power density

1. Introduction

The depletion of non-renewable resources and the environmental consequences associated with their consumption constitute major global challenges in today's technologically advanced society. The excessive use of energy sources leads to serious problems such as climate change, rising global temperatures, and increased greenhouse gas emissions [1–3]. Our dependence on fossil fuels and their detrimental environmental impacts underscore the need to develop alternative technologies and energy sources that can help reconcile ecological conservation with growing energy demands. Furthermore, many regions are increasingly confronted with the challenge of managing wastewater contaminated with hazardous pollutants [4–7]. With the rapid progress of science and technology, human demand for resources has increased steadily. Energy scarcity and environmental pollution caused by the overconsumption of fossil fuels have become global challenges [8–11]. Among emerging bioelectrochemical technologies, Microbial Fuel Cells (MFCs) have garnered considerable attention due to their capacity to directly transform the chemical energy contained in organic substrates into electrical energy via microbial metabolism [12, 13]. MFCs are seen as a promising alternative to fossil fuels because they offer a sustainable and environmentally friendly way to generate energy. They use microorganisms to convert organic waste into bioelectricity, providing a renewable energy source that significantly reduces carbon emissions compared to conventional fuels [14–17]. MFCs employ electrogenic microorganisms that oxidize organic compounds and transfer electrons to an anode, generating electricity while degrading waste materials [12]. This dual function makes MFCs promising systems for waste valorization, wastewater treatment, and bioelectricity production [18]. The fundamental characteristic of microbial activity within MFCs involves electroactive processes [19, 20]. Although these microorganisms produce energy to satisfy their metabolic demands through the oxidative breakdown of carbon-based substrates, they simultaneously release electrons and protons as by-products of these biochemical reactions. The released electrons are then utilized for the generation of electrical energy [21–23]. The anode functions as an electron acceptor, facilitating the oxidation process whereby electrons are liberated from the anodic material. These liberated electrons traverse through an external electrical circuit to reach the cathode, where they participate in reduction reactions with electron acceptors, most commonly molecular oxygen, resulting in the formation of water as the

final product [24, 25]. The various applications of MFC is outlined in Figure 1.

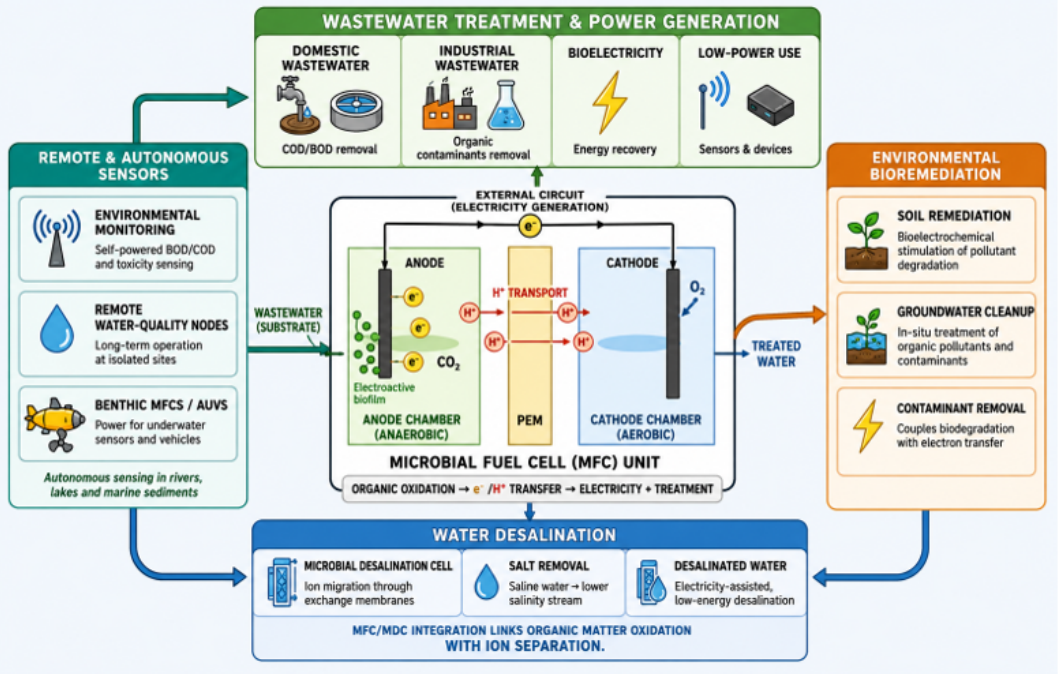

Figure 1. Schematic overview of MFC mechanisms and applications. Electroactive biofilms oxidize biodegradable organic matter in the anode chamber, releasing electrons through an external circuit and protons across the proton exchange membrane, thereby enabling simultaneous wastewater treatment, electricity generation, sensing, bioremediation, and microbial desalination.

Agricultural residues are increasingly explored as substrates for MFCs because they are inexpensive, renewable, and readily available [13, 26]. Cassava processing generates large quantities of peels that are commonly discarded into the environment, causing odor nuisance and environmental pollution [27, 28]. Cassava peels are rich in starch, cellulose, hemicellulose, and fermentable organic compounds, making them suitable feedstock for microbial metabolism and subsequent electricity generation in MFC systems [29, 30]. Previous studies demonstrated that cassava peel extracts can support microbial growth and produce measurable bioelectricity, confirming their suitability as renewable substrates for MFC operation [31, 32]. The previous studies on cassava peel-powered MFCs inoculated with *Bacillus subtilis* is highlighted in Table 1.

Table 1.
Summary of Previous Studes in Cassava Peel-Powered MFCs Inoculated with *Bacillus subtilis*.

Substrate	Inoculum	Key findings	Limitation	Relevance to the current study	References
Cassava wastewater	Indigenous anodic microbial community	Achieved maximum power density of 656.4 $\mu W\,m^{-2}$ and substantial COD removal during cassava wastewater treatment.	Performance strongly depended on organic loading rates and microbial adaptation.	Confirms the suitability of cassava-derived wastes as substrates for bioelectricity generation.	[33]
Organic-rich culture medium	*Bacillus paramycoides*	Demonstrated strong electrogenic properties of *Bacillus* species and efficient extracellular electron transfer during MFC operation.	Did not evaluate lignocellulosic agricultural wastes.	Suggests that *Bacillus* species can serve as efficient anodic biocatalysts in cassava peel-powered MFCs.	[34]
Cassava straw-derived porous carbon anode with organic substrates	Mixed electroactive bacteria	Developed cassava-derived porous carbon anodes that increased power density by approximately 1.59-fold.	Focused on electrode material rather than cassava peel substrate utilization.	Demonstrates the growing importance of cassava biomass in advanced MFC development.	[35]
Sediment organic matter	Indigenous electrogens	Improved power density through electrode modification and enhanced electron transfer pathways.	Did not investigate substrate biodegradation efficiency.	Highlights the importance of electrode optimization for improving cassava peel MFC performance.	[36]
Cassava peel substrate (7.5, 15 g, 22.5 g, 30 g)	*Bacillus subtilis* isolates	Evaluates voltage, current, and power generation using varying cassava peel concentrations inoculated with different *Bacillus subtilis* isolates to identify the most efficient bioelectrogenic strain.	Study ongoing.	Extends previous studies by integrating cassava peel valorization with isolate-specific assessment of *Bacillus subtilis* performance in MFCs.	Current study

The performance of MFCs depends largely on the electrogenic potential of the inoculated microorganisms. Although mixed microbial consortia dominated by *Geobacter* and *Shewanella* species are widely reported, recent studies have shown increasing interest in Gram-positive bacteria such as *Bacillus subtilis* due to their metabolic versatility, environmental adaptability, biofilm formation ability, and extracellular electron transfer capacity [37]. *B. subtilis* can oxidize complex organic substrates and transfer electrons either directly through membrane-associated redox proteins or indirectly through soluble electron mediators. Furthermore, its endospore-forming capability enhances its survival under fluctuating environmental conditions, improving long-term MFC stability [38]. Recent investigations have further demonstrated the electrogenic capability of *B. subtilis* in diverse MFC configurations. The organism has been successfully employed in chlorophenol degradation-coupled electricity generation systems, where optimization of pH and inoculum size significantly improved power output. Likewise, *B. subtilis* endospores have been utilized as dormant biocatalysts in wearable MFC systems, demonstrating their potential for sustained bioelectricity generation under nutrient activation conditions. These findings emphasize the broad applicability of *B. subtilis* as an efficient electrogen in bio-electrochemical systems [39]. Nigeria is among the world's leading cassava producers, generating substantial quantities

of cassava processing wastes annually. Despite the abundance of this biomass resource, utilization for bioenergy production remains limited. Integrating cassava peel substrates with *B. subtilis*-driven MFCs presents an environmentally sustainable strategy for converting agro-waste into electricity while reducing environmental burden. However, information on the electrochemical performance of cassava peel-driven MFCs inoculated specifically with *B. subtilis* remains limited. However, studies specifically combining cassava peel substrate with *Bacillus subtilis* isolates remain limited. Consequently, the present study addresses an important research gap by investigating the comparative bioelectrogenic performance of *Bacillus subtilis* isolates using cassava peel as the primary carbon source for sustainable bioelectricity generation. Consequently, this research supports SDG 6 (Clean Water and Sanitation), SDG 7 (Affordable and Clean Energy), and SDG 12 (Responsible Consumption and Production) by demonstrating the valorization of cassava peel waste as a sustainable substrate for bioelectricity generation in microbial fuel cells, thereby promoting waste-to-energy conversion, resource recovery, and environmentally sustainable waste management.

2. Materials and methods

The materials and equipment used in this research consist of fresh cassava peels, nutrient agar, NaCl, phosphate buffer solution, distilled water, *Bacillus subtilis* isolate, super glue, cotton wool, masking tape, and a sterile glove. The equipment used consists of an autoclave, incubator, analytical balance, glass beakers, sterile containers, two 850 mL bottles serving as containers for the anode and cathode, a salt bridge, PVC pipes, a union, carbon rods as electrodes, a digital multi-meter, and copper wires.

2.1 Cassava Peels Collection

Cassava peels were collected from local cassava processing sites located within the Lokoja metropolis. The samples were transported to the laboratory in clean polyethylene bags to prevent contamination from taking place. The cassava peels were thoroughly washed to remove any adhering soil and dirt, and then dried in the sun for a period of four days. The dried peels were ground into a fine powder by means of a grinder.

2.2. Microbial Samples

Soil samples were collected from refuse dumps in Felele, Lokoja, Nigeria. Sterile spatulas were used to collect approximately 200 g of soil samples from each site at a depth of 5–10 cm. Samples contained in sterile polyethylene bags were labelled and transported to the laboratory for microbial isolation.

2.3 Proximate Analysis of Cassava Peel

The nutritional content of cassava peel was analyzed by determining its proximate composition. The parameters determined included cellulose content, lignin, hemi-cellulose, moisture content, ash, crude lipid, crude fibre, crude protein, carbohydrate content. The methods employed were those outlined by [40, 41].

2.4 Morphological and Biochemical Identification of *Bacillus subtilis*

The soil samples were serially diluted in sterile saline, and aliquots were inoculated onto nutrient agar plates using the spread plate technique. Plates were incubated at 37 °C for 24–48 hours. The cultural and microscopic characteristics of the bacterial isolate recovered from soil samples were examined using standard microbiological procedures. Colonies developed on nutrient agar after incubation appeared cream to whitish in colour, dry, opaque, and irregular in shape with undulate margins and a slightly raised elevation. The colonies exhibited a rough surface texture typical of *Bacillus* species [42, 43].

2.5 Construction and Experimental Setup of the MFC

A dual-chamber MFC was constructed to assess the bioelectricity generation potential of cassava peel waste that had been inoculated with *Bacillus subtilis*. The system comprised two 850 mL plastic bottles, one serving as the anode chamber and the other as the cathode chamber. The chambers were connected via a salt bridge, which was prepared using 1.62 g of agar and 0.54 g of sodium chloride, thereby facilitating proton transfer between the compartments while maintaining the separation of electrolytes. The anode chamber was filled with a mixture of 7.5 g, 15 g, 22.5 g, and 30 g of powdered cassava peel in 750 mL of distilled water containing cassava peel slurry, and was inoculated with isolates of *Bacillus subtilis* under anaerobic conditions. The cathode chamber held 750 mL of phosphate buffer solution at pH 7.0 and was kept under aerobic conditions to serve as the terminal electron acceptor. Due to their durability, conductivity, and compatibility with microbial biofilms, carbon rods were used as electrodes. The electrodes were arranged so that the anode was completely submerged in the cassava peel slurry and the cathode was immersed in the buffer solution. The insulated copper wires, which connected the electrodes externally, were then attached to a digital multi-meter for the measurement of voltage and current. The arrangement enabled the monitoring of electron transfer from microbial metabolism at the anode to the cathode through the external circuit [32, 40]. The nature of MFC set-up and configuration based on this study is illustrated in Figure 2.

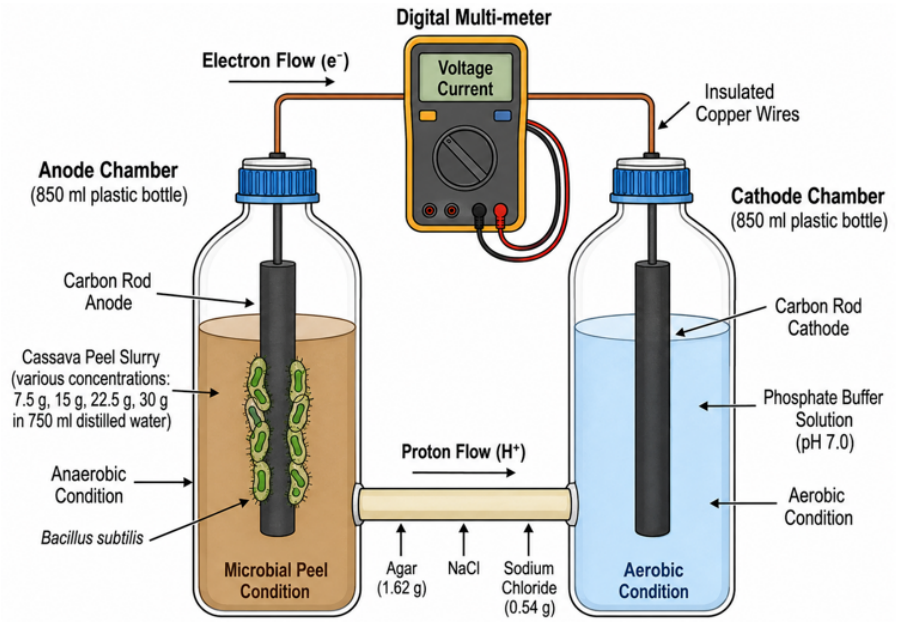

Figure 2. The nature of MFC set-up and configuration based on this study.

2.6 Operation of the MFC and Data Collection

The MFC was operated under anaerobic conditions in the anode chamber and aerobic conditions in the cathode chamber. The anode chamber is sealed tightly under anaerobic conditions to prevent oxygen from penetrating, thereby promoting microbial respiration on the cassava peel substrate. In contrast, the cathode chamber was exposed to atmospheric oxygen to facilitate reduction reactions, thereby ensuring continuous electron flow through the external circuit. A calibrated digital multi-meter was connected to the copper wires linking the electrodes to monitor the electrical output on a daily basis. The generated current was calculated using Ohm's Law. The voltage 'V', the generated current 'I', and the area of the anode 'A' were used in Equations (1–3) to calculate power and current densities [44].

$$V = IR \tag{1}$$

$$PD = \frac{V^2}{A \times R} \tag{2}$$

$$CD = \frac{V}{A \times R} \tag{3}$$

where PD refers to power density ($\mathrm{mW/m^2}$), CD refers to current density ($\mathrm{mA/m^2}$), V is the output voltage (mV), A is the area of anode ($\mathrm{m^2}$), and R is the external resistance. Graphical presentations of voltage, current, and power output trends were generated using Microsoft Excel.

2.7 Description of Study Area

The study took place in Lokoja, the capital of Kogi State, Nigeria, located at the confluence of the Niger and Benue rivers. The city is located within the

Guinea savannah ecological region and has a tropical climate characterised by a rainy season from April to October and a dry season from November to March. Lokoja was selected as the research site due to its status as a significant hub for cassava processing, where numerous small- and medium-sized enterprises manufacture gari, fufu, and starch. Cassava peel waste is frequently discarded in unregulated open dumpsites in large quantities. The abundance of cassava peels, combined with the presence of microbial communities adapted to degrading fibrous plant matter, renders Lokoja an ideal location for sourcing both feedstock and electroactive bacterial strains for this research.

2.8 Control Experiments

Experimental treatments and control setups were included to validate the contribution of microbial metabolism to electricity generation. A negative control, comprising cassava peel substrate that was not inoculated with bacteria, was used to account for abiotic electron transfer. A sterile control was also prepared by inoculating sterilized cassava peel with an autoclaved bacterial suspension, which served as a baseline to exclude any residual electrochemical activity. The comparison of results from experimental and control groups enabled the determination of the specific role of *B. subtilis* in enhancing bioelectricity generation.

2.9 Biosafety and Ethical Considerations

Due to *Bacillus subtilis* being an opportunistic human pathogen, strict biosafety measures were implemented throughout the study. All laboratory work was conducted by researchers under Biosafety Level 2 (BSL-2) conditions, wearing sterile gloves, lab coats, and protective masks. Microbial residues were sterilised by autoclaving prior to disposal, in accordance with institutional guidelines for waste management and culture disposal. The institutional research and ethics committee approved the study, thereby guaranteeing compliance with safety standards and responsible handling of microbial isolates.

3. Results and Discussion

3.1 Proximate Composition of Cassava Peel Substrate

Cassava peel is increasingly recognized as a valuable lingo-cellulosic biomass for sustainable bioenergy production because of its appreciable cellulose, hemicellulose, and fibre contents. The compositional analysis of the cassava peel used in this study showed 32% cellulose, 16% hemicellulose, 13% lignin, 19% crude fibre, 13% moisture, 3% protein, and 2% ash. These components strongly influence substrate biodegradability, microbial metabolism, and electricity generation in MFCs [45]. The high cellulose content (32%) indicates that cassava peel possesses substantial quantities of fermentable carbohydrate capable of supporting microbial growth and electron production. Cellulose serves as the principal structural

polysaccharide in plant biomass and acts as a long-term carbon source during microbial degradation [46]. Recent studies have reported that cellulose-rich agricultural wastes are highly suitable for bio-electrochemical systems because microbial hydrolysis of cellulose releases glucose and reducing equivalents needed for extracellular electron transfer. MFC technologies utilizing lingo-cellulosic biomass have therefore gained attention as environmentally sustainable alternatives for renewable energy generation. The hemicellulose content (16%) further enhances the biodegradation potential of cassava peel [47]. Hemicellulose is more amorphous and less resistant to hydrolysis than cellulose, making it easier for microorganisms to degrade during the initial stages of metabolism. Its breakdown releases sugars such as xylose and arabinose, which can be utilized by electroactive microorganisms for bioelectricity production [48]. Recent investigations have shown that lingo-cellulosic substrates containing moderate hemicellulose levels improve substrate digestibility and accelerate microbial fermentation processes in MFCs and other bioenergy systems [49]. The lignin content of 13% suggests moderate recalcitrance of the cassava peel biomass. Lignin forms a protective matrix around cellulose and hemicellulose, thereby limiting microbial accessibility and slowing substrate degradation. However, the lignin value obtained in this study is relatively lower than that reported for several other agricultural residues, indicating better biodegradability potential [50]. Moderate lignin concentration may also contribute to sustained and gradual release of fermentable substrates during prolonged microbial activity. Recent studies have emphasized that pre-treatment technologies such as alkaline hydrolysis, steam explosion, enzymatic hydrolysis, and biological pre-treatment are effective in disrupting lignin structures and improving cellulose accessibility for microbial metabolism [51]. The crude fibre content of 19% confirms the fibrous lingo-cellulosic nature of cassava peel. Crude fibre contributes to gradual biodegradation and sustained electron release in microbial fuel cells. Although excessive fibre may reduce rapid hydrolysis, moderate fibre levels can support long-term stability of microbial activity and voltage generation [52]. Similar findings have been reported in recent bioenergy studies where agricultural residues with moderate fibre content exhibited improved reactor stability and prolonged electricity generation.

Moisture content (13%) is important because water supports enzymatic reactions, nutrient transport, and microbial metabolism. The moderate moisture level observed suggests favourable storage stability and reduced spoilage before utilization [53]. Excessive moisture often accelerates substrate deterioration during storage, whereas very low moisture can limit microbial accessibility to nutrients. Recent bio-electrochemical studies have shown that optimized moisture content improves substrate hydrolysis and enhances microbial colonization in biomass-fed MFC systems [54]. The protein content of 3% indicates the presence of nitrogenous compounds required for microbial growth and enzyme synthesis. Although relatively low, this protein level is beneficial because it can support bacterial proliferation without causing excessive ammonia accumulation that may inhibit microbial activity. Nitrogen availability is important for the formation of stable anodic biofilms and effective microbial metabolism during electricity generation [55]. The ash content of 2% represents the inorganic mineral fraction of

cassava peel. Low ash content is advantageous because excessive mineral accumulation may interfere with microbial activity and increase reactor fouling [56]. The ash fraction may nevertheless provide trace minerals such as calcium, magnesium, and potassium required for enzymatic activities and microbial growth. Recent investigations on Nigerian lignocellulosic biomass similarly reported that cassava peels contain relatively low ash levels suitable for renewable energy applications and biofuel production [57].

In conclusion, the compositional profile of cassava peel demonstrates its suitability as a promising substrate for sustainable bioelectricity production. The combination of high cellulose and moderate hemicellulose contents provides sufficient biodegradable organic matter for microbial metabolism, while the moderate lignin and fibre fractions support sustained biodegradation and prolonged energy generation. Recent studies on cassava peel valorization further confirm that cassava waste can serve as an effective renewable feedstock for MFCs, bioethanol production, wastewater treatment, and other circular bio-economy applications [58].

Table 2.
Characteristics of the Cassava Peel Waste (CPW) used in the current study.

Components	Percentage Composition (%)
Cellulose	32
Hemi-cellulose	16
Lignin	13
Crude Fibre	19
Moisture	13
Protein	3
Ash	2

3.2 Morphological and Biochemical Characterization of *Bacillus subtilis* Isolate

3.2.1 Morphological Characteristics of the Isolate

The cultural and microscopic characteristics of the bacterial isolate recovered from soil samples were examined using standard microbiological procedures. Colonies developed on nutrient agar after incubation appeared cream to whitish in colour, dry, opaque, and irregular in shape with undulate margins and a slightly raised elevation. The colonies exhibited a rough surface texture typical of *Bacillus* species [59]. Microscopic examination following Gram staining revealed Gram-positive rod-shaped cells arranged singly and occasionally in short chains. Endospore staining further confirmed the presence of centrally located endospores characteristic of *Bacillus subtilis* [60]. The isolate was motile and demonstrated active movement during motility testing. These phenotypic characteristics were consistent with descriptions reported for *Bacillus subtilis* in previous studies. Based on the cultural morphology, Gram reaction, endospore formation, motility pattern, and biochemical profile, the isolate was identified as *Bacillus subtilis*. The isolate demonstrated characteristics commonly reported for

electrogenic *B. subtilis* strains used MFC application particularly its strong enzymatic activities and biofilm-forming potential that support extracellular electron transfer and bioelectricity generation [61].

Table 3.
Morphological Characteristics of the isolated *Bacillus subtilis.*

Characteristics	Observation	Inference
Colony colour	Creamy white	Typical *Bacillus* appearance
Colony shape	Irregular	Characteristic of *Bacillus* spp.
Colony margin	Undulate	Consistent with *B. subtilis*
Elevation	Slightly raised	Typical growth morphology
Surface texture	Rough Dry	Characteristic feature
Opacity	Opaque	Positive
Gram reaction	Gram-positive	Presence of thick peptidoglycan wall
Cell Morphology	Single cells/short chains	Bacillary form
Arrangement	Single cells/short chains	Typical *Bacillus* pattern
Endospore Formation	Present	Endospore former
Motility	Positive	Motile isolate

3.2.2 Biochemical Characteristics of the Isolate

The biochemical profile of the isolate was evaluated to confirm its identity. The isolate tested positive for catalase activity, indicating the production of catalase enzyme capable of decomposing hydrogen peroxide into water and oxygen. Positive reactions were also obtained for citrate utilization, starch hydrolysis, motility, and Voges–Proskauer (VP) reaction, indicating acetoin production during glucose metabolism. The isolate showed negative reactions for indole production and methyl red test, while oxidase activity was positive. The observed biochemical characteristics corresponded with standard identification profiles of *Bacillus subtilis* [62].The purple rod appearance confirms that the isolate is Gram-positive due to the thick peptidoglycan layer in the cell wall which retains crystal violet during staining. *Bacillus subtilis* is characteristically reported as Gram-positive rods in microbiological identification studies. Catalase positivity indicates the organism produces catalase enzyme that decomposes hydrogen peroxide into water and oxygen [63]. Bubble formation confirms aerobic metabolism, a common feature of *B. subtilis*, enabling survival under oxidative stress condition. The positive oxidase reaction indicates the presence of cytochrome c oxidase involved in electron transport and aerobic respiration. Purple coloration confirms active oxidative metabolism and efficient electron transfer mechanisms [64]. Although oxidase reactions may vary among *Bacillus* species, several studies reported oxidase-positive responses in environmental isolates of *B. subtilis* associated with biofilm formation and extracellular electron transfer in MFC systems [65]. Positive citrate utilization shows the organism can use citrate as the sole carbon source through citrate

permease activity. The blue colour results from alkaline by-products causing bromothymol blue indicator change. *B. subtilis* commonly exhibits metabolic flexibility including citrate metabolism. The clear halo demonstrates extracellular amylase production which hydrolyses starch into simpler sugars. *B. subtilis* is known for high amylase secretion and industrial enzyme production, making starch degradation a major identifying feature [66]. Diffuse growth indicates motility resulting from peritrichous flagella. Motility enhances substrate colonization, biofilm formation, and electrode attachment in microbial fuel cells. Motile behavior of *B. subtilis* contributes to its adaptability in bioelectrochemical systems. VP positivity indicates acetoin production via the butanediol fermentation pathway. Red colour development confirms acetoin accumulation, which is frequently reported in *B. subtilis* biochemical characterization [67]. Negative methyl red suggests the organism does not produce stable mixed-acid fermentation end products and instead follows neutral product fermentation pathways. This aligns with the VP-positive characteristic commonly reported for *B. subtilis*. The negative indole test indicates inability to degrade tryptophan into indole due to absence of tryptophanase enzyme. Most *B. subtilis* isolates are reported as indole negative. Absence of pink colour indicates inability to hydrolyse urea to ammonia and carbon dioxide [68]. Urease negativity differentiates *B. subtilis* from some urease-positive *Bacillus* species. Observation of green spores confirms endospore formation, one of the defining characteristics of *Bacillus* species [69]. Endospore production enables resistance to environmental stress, nutrient limitation, and harsh conditions, making *B. subtilis* suitable for environmental and bioenergy applications. The biochemical profile obtained strongly supports the identification of the isolate as *Bacillus subtilis* [70]. The combination of Gram-positive rods, catalase positivity, motility, starch hydrolysis, citrate utilization, and endospore formation agrees with classical identification schemes and recent reports on *B. subtilis* isolated from environmental samples and bioelectrochemical systems [71]. The positive oxidase response further suggests active electron transport capability, which may contribute to extracellular electron transfer during microbial fuel cell operation. Furthermore, starch hydrolysis and citrate utilization indicate broad metabolic capability, enabling the bacterium to utilize complex organic substrates such as cassava peel biomass. Endospore formation provides environmental resilience and prolonged viability during MFC operation [72]. These properties collectively explain the suitability of *Bacillus subtilis* for sustainable bioelectricity generation and biomass conversion applications.

Table 4.
Biochemical Characteristics of the Isolated *Bacillus subtilis.*

Biochemical Test	Observation	Results
Gram Staining	Purple rods	Positive (+)
Catalase Test	Bubble production	Positive (+)
Oxidase Test	Purple coloration	Positive (+)
Citrate Utilization	Blue coloration	Positive (+)
Starch Hydrolysis	Clear halo around colony	Positive (+)
Motility test	Diffuse growth	Positive (+)
Voges–Proskauer test	Red colour development	Positive (+)
Methyl red test	No red colour	Negative (−)
Indole test	No red ring	Negative (−)
Urease	No pink coloration	Negative (−)
Endospore staining	Green Spores Observed	Positive (+)

3.3 Performance of the Microbial Fuel Cells (MFCs)

Figure 3a, b and c illustrates the daily voltage, power, and current generation profiles of MFCs inoculated with different concentrations of *Bacillus subtilis* using cassava peel substrate. The results demonstrate that substrate concentration significantly influenced the bio-electrochemical performance of the MFCs, affecting microbial metabolism, extracellular electron transfer, and electricity generation efficiency.

In Figure 3a, the voltage generation profile revealed fluctuating electrical outputs across the different substrate concentrations throughout the 14-day experimental period. Among all treatments, the 30 g substrate concentration exhibited the earliest and highest voltage peak, reaching approximately 0.72 mV on day 2 before declining rapidly afterward. This initial sharp increase suggests rapid microbial adaptation and accelerated utilization of readily biodegradable organic compounds present in the cassava peel substrate. Similar observations have been reported in recent studies where high substrate concentrations stimulated rapid microbial metabolic activity and enhanced initial electron release during the early stages of MFC operation [73]. The rapid decline in voltage observed after day 2 in the 30 g treatment may be attributed to substrate depletion, accumulation of metabolic by-products, proton build-up, or inhibition caused by excessive organic loading. High substrate concentrations can create unstable anodic environments that reduce microbial efficiency over time. Recent reports on lignocellulosic biomass-fed MFCs showed that excessive organic matter may increase internal resistance and inhibit long-term voltage stability due to substrate overloading and microbial stress [74]. The 22.5 g treatment demonstrated a delayed but relatively stable voltage peak around day 8, reaching approximately 0.70 mV before gradually decreasing. This suggests that moderate substrate concentration provided a more balanced nutrient supply that sustained microbial metabolism over a longer period. The gradual decline after the peak may indicate progressive exhaustion of fermentable sugars and reduced microbial activity. Similar delayed voltage peaks have been reported in cassava wastewater and agricultural biomass-fed MFCs,

where microorganisms required an adaptation phase before achieving maximum electrogenic activity [75]. The 7.5 g treatment also produced appreciable voltage generation, reaching a peak around day 7 before stabilizing at moderate values. This indicates that lower substrate concentrations may favor prolonged microbial activity and reduced substrate inhibition. In contrast, the 15 g treatment generated relatively low and stable voltage throughout the study period, suggesting insufficient substrate availability or less efficient microbial electron transfer under those conditions. The observed variations in voltage generation align with previous findings that substrate concentration, microbial adaptation, and lignocellulosic composition strongly influence electrogenic performance in microbial fuel cells. Cassava peel contains cellulose, hemicellulose, and soluble organic compounds that support microbial respiration and extracellular electron transfer [58].

In Figure 3b, power generation profile followed trends similar to those observed for voltage generation because power output is directly dependent on voltage and current production. The 7.5 g treatment exhibited the highest sustained power generation between days 8 and 12, reaching approximately 0.095 mW. This suggests that lower substrate loading provided favorable conditions for efficient electron transfer and reduced internal resistance within the MFC system. Interestingly, although the 30 g treatment generated the highest early voltage peak, its power output rapidly declined after day 2. This indicates that higher substrate concentration alone does not guarantee sustained power generation. Excessive substrate loading may lead to oxygen diffusion limitations, accumulation of toxic metabolites, or disruption of anodic biofilm stability, thereby reducing overall energy conversion efficiency. Similar observations have been documented in recent MFC studies where excessive organic loading caused reduced coulombic efficiency and unstable power production [74]. The moderate power output observed in the 22.5 g treatment suggests that intermediate substrate concentrations support balanced microbial growth and gradual substrate degradation. The low power generation recorded in the 15 g treatment further indicates suboptimal microbial activity and limited electron recovery. Recent studies on cassava peel and cassava wastewater-fed MFCs have reported that power generation is strongly affected by substrate composition, microbial community structure, pH stability, and electrode conductivity. Pretreatment methods that improve cellulose hydrolysis and microbial accessibility have been shown to enhance power density in lignocellulosic biomass-fed MFCs [76].

In Figure 3c, current generation profile demonstrated substantial differences among the substrate concentrations. The 30 g treatment produced the highest current during the early phase of operation, reaching approximately 0.20 mA around day 7 before progressively declining. High current production during the initial stage indicates active microbial oxidation of organic matter and enhanced extracellular electron transfer by *Bacillus subtilis* biofilms [77]. The gradual decline in current after the peak suggests depletion of biodegradable substrates and reduced metabolic activity. In microbial fuel cells, current generation is directly associated with microbial respiration and electron transfer efficiency. As fermentable organic compounds become exhausted, microbial metabolism

decreases, leading to lower current production. Similar trends have been reported in recent studies involving agricultural waste-fed MFCs and cassava wastewater systems [78]. The 7.5 g treatment exhibited delayed but relatively stable current generation between days 8 and 13, suggesting sustained microbial activity under lower substrate concentration. This finding indicates that moderate substrate loading may enhance long-term electrogenic stability and reduce inhibitory effects associated with substrate overloading. The 22.5 g and 15 g treatments produced comparatively lower current outputs, indicating less efficient electron transfer and microbial metabolism. These variations may be associated with differences in biofilm development, substrate hydrolysis rate, and microbial adaptation to the lignocellulosic cassava peel substrate. Recent literature has shown that Biofilm Formation is essential for stable current generation because electroactive biofilms facilitate direct electron transfer from microbial cells to the anode surface [79]. The performance observed in this study therefore suggests that *Bacillus subtilis* possesses appreciable electrogenic potential capable of supporting bioelectricity generation from cassava peel biomass.

Overall, the results indicate that substrate concentration significantly influenced voltage, power, and current generation in cassava peel–powered microbial fuel cells inoculated with *Bacillus subtilis*. Higher substrate concentrations promoted rapid initial electricity generation but reduced long-term stability, whereas lower concentrations supported more sustained electrogenic activity [80]. The findings further demonstrate the suitability of cassava peel as a renewable lignocellulosic substrate for microbial fuel cell applications. The observed electrical outputs are consistent with recent studies emphasizing that agricultural wastes such as cassava peels can serve as sustainable feedstocks for bioelectricity generation, waste valorization, and environmental management. Although the power outputs remain relatively low for large-scale application, optimization of reactor design, substrate pretreatment, electrode materials, and microbial engineering may substantially improve MFC performance in future studies.

3.4 Power Density of Cassava Peel Substrate

Figure 5 illustrates the variation in power density (mW/m^2) of MFCs operated with different cassava peel substrate concentrations (7.5 g, 15 g, 22.5 g, and 30 g) over a 7-day incubation period. The overall trend indicates that increasing substrate concentration enhanced bioelectricity generation, with the 30 g treatment producing the highest power density throughout the experimental period. At the initial stage (days 1–3), all treatments produced relatively low power densities, suggesting a lag phase associated with microbial acclimatization and early biofilm formation on the anode surface. However, the 22.5 g and 30 g treatments showed faster increases in power generation compared with 7.5 g and 15 g treatments. This observation agrees with recent studies reporting that higher organic loading provides more biodegradable carbon sources for microbial metabolism and extracellular electron transfer (EET), thereby improving electron flow to the anode. From day 4 onward, a sharp increase in power density was observed, especially in the 22.5 g and 30 g reactors. The 30 g treatment increased

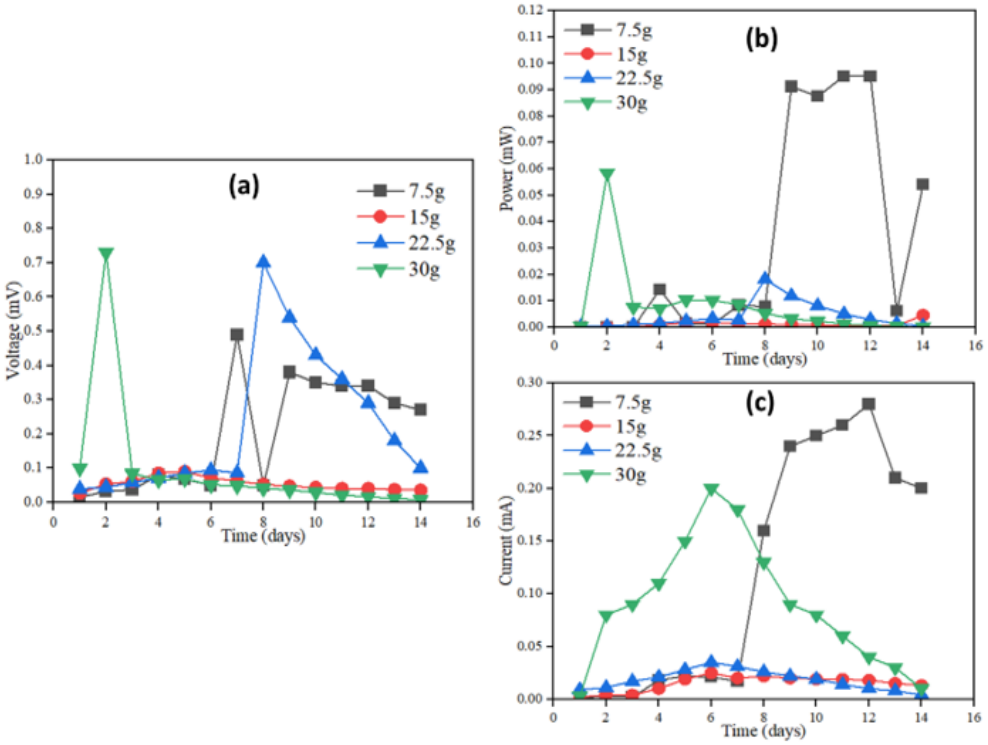

Figure 3. Daily performance: (a) voltage generation (mV) of MFCs inoculated with different *B. subtilis* isolates, (b) power generation of MFCs inoculated with different *B. subtilis* isolates, and (c) current generation (mA) of MFCs inoculated with different *B. subtilis* isolates.

steadily from approximately 7.2 mW/m^2 on day 4 to about 17.8 mW/m^2 on day 7, representing the highest performance among all treatments. This suggests that the microbial consortium, likely dominated by electroactive organisms such as *Bacillus subtilis*, efficiently utilized the cassava peel substrate for electron production. Recent studies have shown that lingo-cellulosic substrates such as cassava peels contain fermentable carbohydrates, cellulose, and hemicellulose which support prolonged microbial activity and improved bioelectricity production in MFCs [57]. The superior performance of the 30 g treatment may also be attributed to enhanced conductivity and increased availability of soluble organic compounds released during substrate degradation. Recent literature indicates that optimal substrate loading improves microbial respiration, promotes thicker electroactive biofilms, and reduces substrate limitation during electricity generation. The 22.5 g treatment also demonstrated substantial power generation, reaching approximately 12.5 mW/m^2 on day 6 before slightly declining on day 7. The slight decrease may indicate substrate depletion, accumulation of metabolic by-products, proton accumulation, or increased internal resistance within the MFC chamber. Similar reductions in later operational stages have been reported in MFC studies where excessive metabolite accumulation inhibited electron transfer efficiency. In contrast, the 7.5 g and 15 g treatments generated significantly lower power densities throughout the experiment. Their lower performance suggests insufficient organic matter availability for sustained microbial metabolism and electron release. Although the 15 g treatment showed a temporary increase around day 5, the subsequent decline indicates instability in substrate utilization or weaker biofilm development compared with higher substrate concentrations.

This aligns with findings that low substrate concentrations often limit bacterial respiration and reduce anodic electron transfer rates in MFC systems [34]. The gradual increase in power density observed in all treatments reflects successful adaptation of electroactive microorganisms and progressive biofilm maturation on the electrode surface. Biofilm development is a critical factor influencing MFC efficiency because it facilitates direct electron transfer between microbial cells and the anode. Recent reviews have emphasized that stable and conductive biofilms significantly improve power density and columbic efficiency in waste-fed MFCs [81]. Overall, the graph demonstrates that increasing cassava peel concentration positively influenced MFC performance, with 30 g producing the optimum power density. The findings support recent research highlighting cassava peel as a promising low-cost lingo-cellulosic substrate for sustainable bioelectricity production and waste valorization.

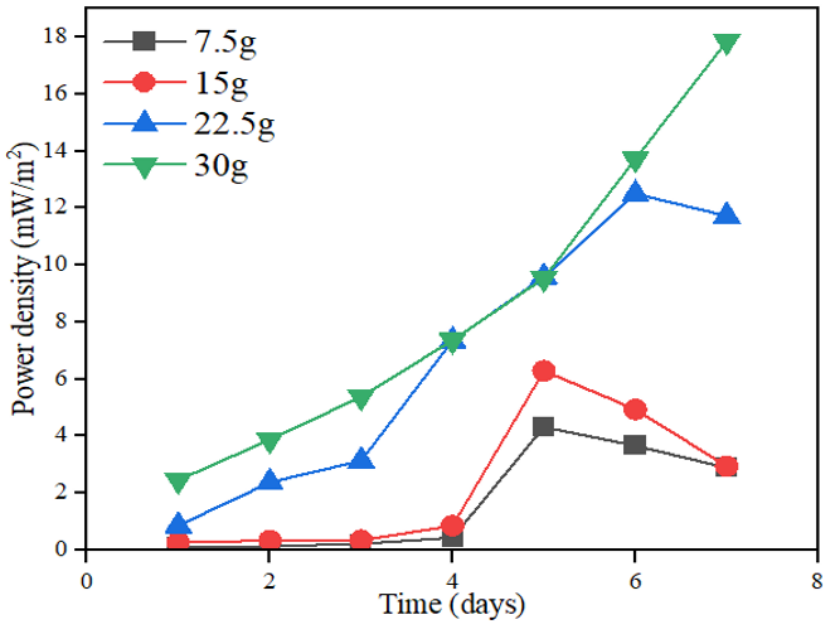

Figure 4. Variation in power density (mW/m ^{2}) of MFCs operated with different cassava peel substrate concentrations.

4. Challenges and Future Perspectives

Despite the growing interest in utilizing cassava peel–powered MFCs inoculated with *Bacillus subtilis* for sustainable bioelectricity generation, several technical, operational, and economic limitations still hinder large-scale application. One major challenge is the relatively low power output generated by cassava peel–based MFCs compared to conventional energy systems [82]. Although cassava peel contains abundant biodegradable organic matter, the complex lingo-cellulosic structure of the substrate often limits microbial degradation and electron release. The presence of lignin and crystalline cellulose reduces substrate bioavailability, thereby decreasing electron transfer efficiency and voltage generation. Another challenge is the limited extracellular electron transfer (EET) capability of many non-model electroactive microorganisms [83]. While EET occurs in *Bacillus subtilis*,

its electron transfer efficiency is generally lower than that of highly electroactive species such as *Geobacter sulfurreducens* and *Shewanella oneidensis* [84]. This limitation affects current density, coulombic efficiency, and overall bioelectricity production. Biofilm instability on the anode surface also constitutes a significant operational challenge. Efficient electricity generation in MFCs depends on stable and conductive microbial biofilms [85]. However, fluctuations in pH, temperature, substrate concentration, and nutrient availability may weaken biofilm formation and reduce microbial metabolic activity. Poor adhesion of bacterial cells to electrode surfaces can further impair electron transport [86]. Internal resistance within the MFC system is another critical issue affecting performance. High resistance may arise from electrode materials, membrane limitations, electrolyte conductivity, and long electrode spacing. These factors reduce electron flow efficiency and energy recovery [87]. In many laboratory-scale systems, costly electrode materials and proton exchange membranes also increase operational expenses and limit commercialization potential. Cassava peels obtained from different processing sources may vary in moisture content, carbohydrate composition, cyanogenic glycosides, and organic load. Such inconsistencies can influence microbial metabolism and lead to irregular power generation. In addition, improper pretreatment of cassava peels may result in substrate inhibition or slow biodegradation rates [88]. Scale-up remains a major bottleneck for practical application. Most reported studies on cassava peel–powered MFCs are conducted at laboratory scale under controlled conditions. Translating these systems into pilot-scale or industrial applications involves challenges such as maintaining microbial stability, optimizing reactor configuration, preventing contamination, and ensuring continuous substrate supply. Furthermore, environmental conditions strongly affect system performance [89]. Variations in temperature, dissolved oxygen concentration, salinity, and pH can alter bacterial activity and electron transfer processes. Maintaining optimal operational parameters in real-world conditions may therefore be difficult, especially in rural or resource-limited settings.

Future research should focus on improving the electrochemical efficiency and scalability of cassava peel–powered MFC systems [90]. One promising direction involves the genetic engineering of electroactive microorganisms to enhance electron transfer pathways, biofilm conductivity, and substrate utilization efficiency. Metabolic engineering of Electroactive Microorganisms could improve flavin production, cytochrome expression, and conductive nanowire formation for enhanced electricity generation. Advancements in nanotechnology and material science may also significantly improve MFC performance [91]. The development of low-cost, conductive, and biocompatible electrode materials such as graphene composites, carbon nanotubes, biochar, and metal oxide nanomaterials could reduce internal resistance and enhance microbial attachment [92]. These materials may increase electron transfer rates while lowering operational costs. Pretreatment technologies for cassava peels should also be optimized to improve substrate biodegradability [93]. Physical, chemical, enzymatic, and biological pretreatment methods can enhance hydrolysis of lingo-cellulosic components, thereby increasing fermentable sugar availability and improving microbial metabolism [94]. Future studies should further explore hybrid systems

that integrate MFCs with anaerobic digestion, wastewater treatment, or biogas production. Such integrated bio-electrochemical systems may improve waste management efficiency while simultaneously generating electricity and valuable byproducts [95]. There is also a need for reactor design optimization to enhance scalability and long-term operation. Continuous-flow MFCs, stacked reactors, and modular systems may increase power density and improve applicability for rural electrification and wastewater treatment in developing countries [96]. The use of membrane-less designs and inexpensive locally sourced materials could further promote commercialization. Artificial intelligence and machine learning approaches may provide additional opportunities for optimizing MFC operational parameters such as pH, temperature, hydraulic retention time, and substrate concentration [97]. Predictive modeling could improve system stability and maximize energy recovery efficiency [98]. In addition, future investigations should focus on long-term environmental sustainability assessments, including life cycle analysis, techno-economic evaluation, and carbon footprint estimation. These assessments are essential for determining the feasibility of cassava peel–powered MFCs as alternative renewable energy technologies [99, 100]. Finally, multidisciplinary collaboration among microbiologists, electrochemists, environmental engineers, material scientists, and policymakers will be necessary to advance the commercialization and practical deployment of cassava peel–powered MFCs for sustainable bioelectricity production [101]. The various challenges and future perspectives of the current research can be explained in Figure 5.

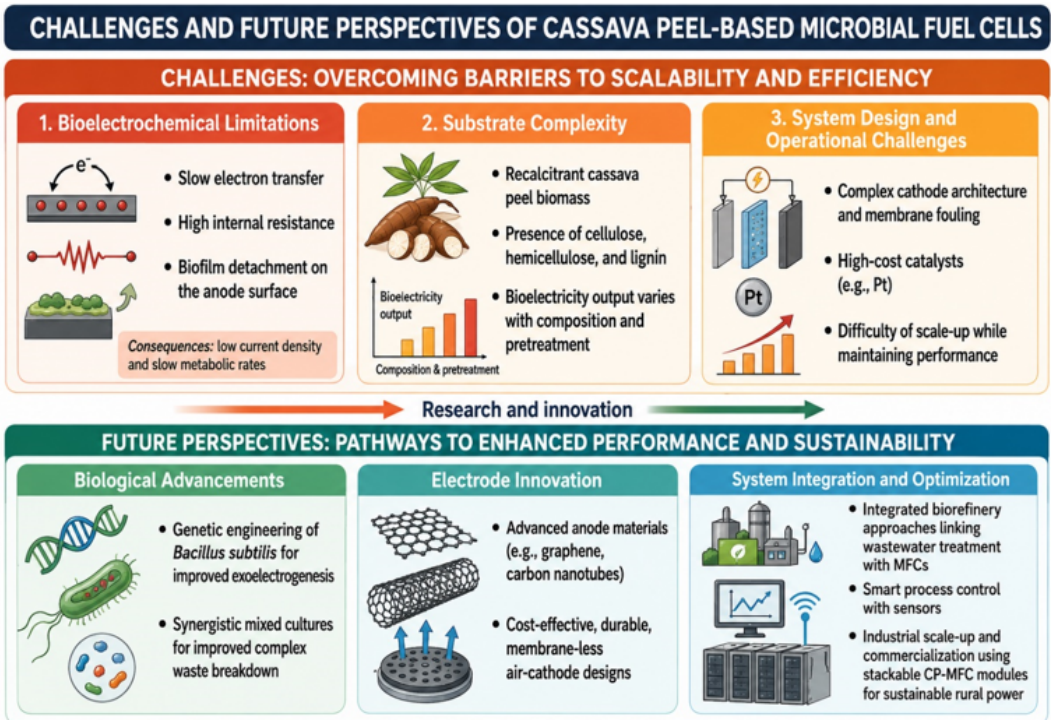

Figure 5. Challenges and Future perspectives of the current study.

5. Conclusion

The evaluation of cassava peel–powered microbial fuel cells inoculated with *Bacillus subtilis* demonstrates the significant potential of agricultural waste valorization for sustainable bioelectricity generation. Cassava peels, which are often discarded as agro-industrial waste, serve as an abundant and renewable carbon source capable of supporting microbial metabolism and electron production in MFC systems. The study highlights the ability of *Bacillus subtilis* to utilize cassava peel substrates for bio-electrochemical energy conversion through microbial oxidation and extracellular electron transfer mechanisms. Variations in substrate concentration influenced voltage generation, indicating that substrate optimization plays a crucial role in enhancing MFC performance. The findings further confirm that microbial fuel cells can simultaneously contribute to renewable energy production and organic waste management. Although several limitations such as low power density, internal resistance, biofilm instability, and scale-up challenges remain, continuous advancements in microbial engineering, electrode materials, reactor configuration, and substrate pretreatment technologies are expected to improve system efficiency and commercial viability. Overall, cassava peel–powered MFC represent an environmentally friendly and sustainable technology with promising applications in renewable energy generation, wastewater treatment, and circular bio-economy development. Continued research and technological innovation will be essential for transforming this emerging bio-electrochemical system into a practical alternative energy solution, particularly in developing countries where cassava waste is abundant and access to clean energy remains limited.

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